CHEMICAL DEFENSE IN ITHOMIINE BUTTERFLIES (NYMPHALIDAE: ITHOMIINAE)

BY

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I dedicate this to

Ransom and Margaret Perkins,

who never lost their love in knowing.

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Abstract of Dissertation Presented to the Graduate School of the University of Florida in Partial Fulfillment of the Requirements for the Degree of Doctor of Philosophy

CHEMICAL DEFENSE IN ITHOMIINE BUTTERFLIES (NYMPHALIDAE: ITHOMIINAE)

By

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Strikingly similar coloration among ithomiine butterflies was the basis of mimicry theory. For mimicry to evolve and be maintained, ithomiines must be protected from insectivores that are sympatric and diurnal, use visual cues in foraging, and associate color pattern with unpalatability. Here, I examine the chemical defense of ithomiine butterflies against two such insectivores: the Spiny Lizard, Sceloporus malachiticus, and the Blue-Crowned Motmot, Momotus momota.

The source of ithomiine chemical defense is believed to be pyrrolizidine alkaloids. I found that heliotrine, a pyrrolizidine alkaloid, was taste-rejected by S. malachiticus and M. momota. While the comparison remains tenative due to molecular differences between heliotrine and pyrrolizidines

found in butterflies, thresholds measured by the "up and down" method were within a range of pyrrolizidine alkaloid quantities that naturally occur in most ithomiine species.

For the most part, pyrrolizidine alkaloids occur only in ithomiine adults and only after adults acquire them from the environment. I examined the unpalatability of one ithomiine species, the tawny-colored Pteronymia fulvescens. Larvae were more unpalatable than mealworms to S. malachiticus and M. momota. Newly emerged adults fed water or a sugar solution were palatable to both predators. Unpalatability occurred in adults after exposure to heliotrine. Wild-captured adult ithomiines were also unpalatable.

Adult ithomiines vary widely in their pyrrolizidine alkaloid concentration. I monitored pyrrolizidine alkaloids in 20 tawny-colored ithomiine species for one year in a single assemblage from Costa Rica. Age and sex had no effect on the amount of pyrrolizidine alkaloids an individual contained. Species differed in content at times, but in no consistent fashion. The month of capture had a significant effect, however. For four consecutive months, pyrrolizidine alkaloid levels were low. After this, levels rose sharply, following the flowering phenology of pyrrolizidine alkaloid-containing plants. A model predicting predation risk showed that variable chemical defense and ithomiine abundance may interact to maintain constant ithomiine survivorship throughout the year. Further analyses showed that predator foraging behavior can alter the benefits of mimicry.

CHAPTER 1
GENERAL INTRODUCTION

"Talk does not cook rice."
Chinese proverb

Ecological chemistry and mimicry theory are probably not the sole refugia of typological thinking in evolutionary biology (Mayr, 1982), but they are significant ones. Mimicry theory, implicitly assuming chemical defense, is historically the principal example of evolution by natural selection (Fisher, 1928; Brower, 1988). Even the earliest discussions of mimicry acknowledge slight within-population variation in morphology upon which natural selection acts to bring about coincident appearance. At the same time, though, chemical defense has been treated as an immutable phenomenon of taxa, including species, genera, families, and even orders (Rothschild and Marsh, 1978). Furthermore, all mimetic species have been divided into two disparate halves: palatable (Batesian mimics) and unpalatable (models or Müllerian mimics).

While variation in chemical defense has been acknowledged several times (review in Brower, 1984), many current studies of butterfly chemical ecology and mimicry assume at least one of three discredited maxims based in typology: (1) all secondary compounds confer protection (e.g., Brown and Francini, 1990; Rothschild et al., 1973);

(2) all defended species are equally protected (e.g., Sillen-Tullberg, 1988); and (3) all individuals or species of predators treat butterflies similarly (Chai, 1990). The resultant species with invariant palatability can be classified as either Batesian or Müllerian mimic/model (Turner, 1987).

These attitudes are tolerated, I believe, because of the difficulty inherent in field/laboratory studies and mathematical models that acknowledge variable chemical defense. In fact, I originally planned to ignore variation in this study of ithomiine (Lepidoptera: Nymphalidae) chemical defense and mimicry. Instead, I proposed to identify a class of compounds found in ithomiines as defensive and then classify all ithomiine species as palatable or unpalatable. Early discussions with L.S. Fink and L.P. Brower convinced me otherwise. They argued that holding to ideas that are clearly untrue, such as species-level chemical protection, can only obscure an understanding of how butterflies and predators interact to shape mimicry. Here, then, is what I believe to be the first exploration of chemical defense and mimicry that expressly attempts to examine the sources and outcome of variable chemical defense in a mimetic complex of several species (see also Ritland, 1991).

Ithomiine butterflies were assumed unpalatable at the onset of mimicry theory (Bates, 1862; Müller, 1879). The chemical responsible for protection was assumed to come from

their solanaceous larval foodplant (Brower and Brower, 1964). This hypothesis was challenged when solanaceous alkaloids were not found in the only ithomiine ever tested, one adult Mechanitis polymnia (Rothschild, 1973). Pyrrolizidine alkaloids were a likely second choice. Ithomiines were attracted to plants containing them (Pliske, 1974), and males used them as pheromone precursors (Edgar et al., 1976).

These initial hypotheses have been tested experimentally. Feeding studies with spiders (Brown, 1984, 1985) and birds (Brower, 1984) confirm ithomiine unpalatability. More species of ithomiines have been chemically analysed and were found to lack solanaceous secondary compounds as adults (Brown, 1985). At the same time, adults of both sexes contained pyrrolizidine alkaloids (Brown, 1984). This is hardly proof of pyrrolizidine alkaloid protection, however. Other compounds may be the cause of unpalatability. Also, although controlled feeding experiments effectively demonstrate ithomiine palatability, some ithomiines were eaten by predators. Furthermore, observations in an ithomiine assemblage suggest that at least one tanager species found ithomiines perfectly edible (Brown and Vasconcellos-Neto, 1976). If there is chemical defense, therefore, it is not a uniform property of ithomiines.

The sources of ithomiine protection and its variation are still unknown. Pyrrolizidine alkaloids have not been tested for negative pharmacological or taste properties with natural vertebrate predators, for instance. Pyrrolizidine

alkaloids are not present in the majority of ithomiine larvae, calling to question the unpalatability of larvae. Finally, pyrrolizidine alkaloids vary widely in ithomiine adults, making pyrrolizidine alkaloid-based unpalatability unlikely for many ithomiines. Why so much variation occurs and how it influences predation remain unanswered. This dissertation will tackle these issues.

Chapter 2 demonstrates that pyrrolizidine alkaloids can act as feeding inhibitors for vertebrate ithomiine predators. The role of pyrrolizidine alkaloids in insect defense has been questioned by many authors. The doubts are twofold. First, because pyrrolizidine alkaloids are slow-acting toxins (Mattocks, 1986), they are unlikely to actuate a conditioned aversion (Aplin et al., 1968; Brower, 1984). Second, pyrrolizidine alkaloids may taste bitter at high concentrations, but at concentrations that are never attained under natural conditions (Glendinning et al., 1991). Using two ithomiine predators and a purified pyrrolizidine alkaloid, heliotrine, I show that at least one pyrrolizidine alkaloid inhibits predation at levels found naturally in ithomiines.

Chapter 3 examines variation in chemical defense through the ithomiine life cycle. If solanaceous alkaloids are not found in ithomiine tissues, immatures and recently emerged adults may be palatable. This is the case with one predator, Nephila clavipes (Arachnida: Araneae; Brown, 1984, 1985). Adult ithomiines are palatable to the spider until they

obtain pyrrolizidine alkaloids (Brown, 1985; Masters, 1990). On the other hand, solanaceous alkaloids are present in the larval gut and many butterfly species manufacture defensive compounds de novo from foodplant precursors (Nahrstedt and Davis, 1981). While such compounds did not defend larvae or recently emerged ithomiine adults against N. clavipes, they may operate against other predators. In this chapter, I extend the study of chemical defense in the ithomiine life cycle to include vertebrate predators.

Chapter 4 explores the source and outcome of pyrrolizidine alkaloid variability for a persistent assemblage of adult ithomiines. I focus on a subset of species that share common elements of color pattern. Variation in ithomiine defense chemistry has been documented for a large number of species (Brown, 1984, 1985, 1987). Ecological sources of variation have been explored on a smaller scale (Trigo and Brown, 1990). These studies hypothesize sex, age, species, and seasonal differences in ithomiine pyrrolizidine alkaloid content that I test here. In addition, I explore the ecological and evolutionary outcome of pyrrolizidine alkaloid variation with a natural predator using feeding experiments and a mathematical model of variable chemical defense and mimicry developed by Brower et al. (1970).

All studies have to limit their scope, so all sources of variation could not be explored here. In Chapter 2 I use just one pyrrolizidine alkaloid, heliotrine. It is similar

to the five pyrrolizidine alkaloids (all isomers) identified in ithomiines (Brown, 1985), but there may be pharmacological differences between these different pyrrolizidine alkaloids (Mattocks, 1986). In Chapter 3, I use a single ithomiine species, Pteronymia fulvescens, to examine at what life stage ithomiines are protected from vertebrate predators. This species has gregarious larvae that may be aposematic, unlike many other ithomiines, and uses a larval foodplant ignored by many ithomiine species. Generalizing trends to all ithomiine species may be tenuous. In Chapter 4, I use a single predator species, the Blue-crowned Motmot (Momotus momota), to assess if species, sexes, and ages of ithomiines differ in palatability and whether the proportion of palatable ithomiines change seasonally. There are countless other predators that will exhibit unique pyrrolizidine alkaloid tolerances. I attempt to restrict discussions to include only the cases I have tested. This, naturally, limits the ultimate power of the dissertation to fully understand the ecological utility of ithomiine chemical defense and its evolutionary role in mimicry. Finally, I assume that the coincident coloration in ithomiine butterflies results, at least in part, from mimicry (see Papageorgis, 1975 for an alternative but not contradictory theory of ithomiine coloration).

In spite of its limitations, I believe this dissertation is a uniquely important study of chemical defense. Ignoring the facile but unrealistic framework of typology has

uncovered a depth of ecological and evolutionary responses to enormous variation in ecological chemistry and mimetic relationships. The result is neither a neat dichotomy nor annihilation of Batesian and Müllerian mimicry. Instead, the pattern that emerges extends the powers of chemical defense and mimicry as protective agents. I will provide the conclusion at the outset: Chemical defense and mimicry can operate effectively in spite of variable protection. Even variable defense greatly increases survival. In ithomiines, this may explain their strikingly similar color patterns. Still, it is one thing to notice and quite another to understand *why*.

CHAPTER 2
PYRROLIZIDINE ALKALOID REJECTION
BY VERTEBRATE PREDATORS

Introduction

Pyrrolizidine alkaloids are found in many insects, including members of the Lepidoptera, Hymenoptera (Boppre, 1990), Orthoptera (Ehmke et al., 1989), and Coleoptera (Pasteels et al., 1990), Insects acquire these secondary compounds from foodplants, plant nectars, plant wounds, seeds, or other insects, or may synthesize them de novo (Boppre, 1986). There is ample evidence linking pyrrolizidine alkaloids to pheromone production. Males metabolize pyrrolizidine alkaloids to volatiles that are used in mate attraction (Meinwald and Meinwald, 1966; Pliske and Eisner, 1969; Boppre, 1990). Pyrrolizidine alkaloids may also serve a defensive function in some species (Rothschild et al., 1973; Boppre, 1986; Eisner and Meinwald, 1987). From the outset, though, the importance of pyrrolizidine alkaloids in insect defense has been debated (Aplin et al., 1968; Brower, 1984).

Evidence in Support of Pyrrolizidine Alkaloid-based Defense

The hypothesis that pyrrolizidine alkaloids act as insect defense compounds is derived from three observations.

First, pyrrolizidine alkaloid-containing insects typically exhibit characteristics associated with unpalatability: aposematism, gregariousness, and slow flight, among others (Poulton, 1908; Kaye, 1914; Brower and Brower, 1964; Brown, 1984; Boppre, 1990). In fact, a few species are unpalatable in choice tests with predators (Brower, 1984). Second, pyrrolizidine alkaloids are found in both males and females. Their presence in both sexes is problematic if the only role of pyrrolizidine alkaloids is their use by males in mate location. Finally, many animals seem to avoid pyrrolizidine alkaloids in non-insect food. Grazing animals avoid pyrrolizidine alkaloid-containing plants, for instance (Bull et al., 1968). Locusts, some lepidopteran larvae, cockroaches, and ants also reject pyrrolizidine alkaloid-tainted plants or artificial feed (Bernays and Chapman, 1977; Bentley et al., 1984; Boppre, 1990).

Direct evidence linking pyrrolizidine alkaloids to insect unpalatability is restricted to two spider species. Nephila clavipes (Arachnida: Araneae), a large orb-weaver, rejects palatable butterflies onto which purified pyrrolizidine alkaloids are painted (Brown, 1985). Ithomiiine butterflies and arctiid moths fed pyrrolizidine alkaloids are also rejected by N. clavipes, yet these same insects denied access to pyrrolizidines are readily eaten (Eisner and Meinwald, 1987; Brown, 1984, 1985; Masters, 1990). This is also the case for the wolf spider Lycosa ceratiola and the moth Utetheisa ornatrix (Arctiidae; Eisner

and Eisner, 1991). The larvae of U. ornatrix are only unpalatable to L. ceratiola when raised on plants containing pyrrolizidine alkaloids.

Evidence Against Pyrrolizidine Alkaloid-based Defense

The effectiveness of pyrrolizidine alkaloids as defensive compounds is questioned because of their pharmacological properties in vertebrates (Aplin et al., 1968; Brower, 1984; Brower and Fink, 1985). Some grazers are not susceptible to pyrrolizidine alkaloids in their diet (Mattocks, 1986). For other vertebrates, pyrrolizidine alkaloids are toxic only when ingested in great amounts and for prolonged periods. In these cases, cell and tissue damage may result over weeks, or pyrrolizidine alkaloids may be carcinogenic after longer exposure (Bull et al., 1968; McLean, 1970; Mattocks, 1986; Scheline, 1991). Even when toxic, though, pyrrolizidine alkaloids may act too slowly for associative learning. Without a prompt negative experience and consequent conditioned avoidance, predators may never associate the taste of pyrrolizidine alkaloids or other substances with their negative physiological effects. If this is the case, predators would not discriminate between insects with and without pyrrolizidine alkaloids, making them ineffective as defense compounds (Brower, 1984).

Even in the absence of such doubts, a relationship between pyrrolizidine alkaloids and insect unpalatability is

not proof of causality; pyrrolizidines often occur with other secondary compounds (Aplin et al., 1968; Aplin and Rothschild, 1972). For example, danaine butterflies contain both cardenolides and pyrrolizidine alkaloids (Brown, 1984; Ackery and Vanewright, 1984; Kelly et al., 1987). Cardenolides are emetic and if present in sufficient quantity, deter predation by conditioned aversion (Brower et al., 1982; Brower and Fink, 1985; Brower et al., 1988). While cardenolides are present in suprathreshold quantities, feeding experiments suggest the pyrrolizidine alkaloid content of overwintering monarchs may be too low to confer protection against their rodent predators (Glendinning et al., 1991). Thus, even when pharmacologically active, pyrrolizidine alkaloids may not be present in sufficient quantity in insects to confer protection. If so, the insect's unpalatability must be attributed to unrelated compounds.

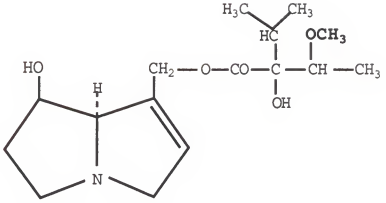
Clarifying the Role of Pyrrolizidine Alkaloids in Insect Defense

In summary, direct evidence that pyrrolizidine alkaloids protect insects against predation is restricted to two spider species. For these predators, feeding experiments demonstrate insect unpalatability and only in the presence of pyrrolizidine alkaloids. I attempt to clarify the role of pyrrolizidine alkaloids in insect defense against vertebrate predators. I do this using vertebrate insectivores likely to

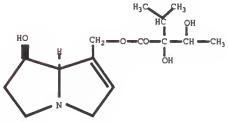
prey upon insects that contain pyrrolizidines. Further, I use experiments that explicitly test pyrrolizidine alkaloid unpalatability, not insect unpalatability. At the same time, these experiments quantify the amount of pyrrolizidine alkaloid necessary to inhibit predation. I attempt to compare the measured thresholds to levels in insects. Finally, I demonstrate that pyrrolizidine alkaloids induce a negative stimulus in the short term, so that immediate or conditioned avoidance of prey may operate.

Here, I use palatable moths and heliotrine, a pyrrolizidine alkaloid found in Heliotropium spp. (Boraginaceae; Mattocks, 1986). Heliotrine is structurally similar to the pyrrolizidine alkaloids found in many insects (Brown, 1985; Figure 2-1) but can be purchased in pure form (Sigma Chemical Co.). I chose two insectivores: the Spiny Lizard Sceloperus malachiticus (Reptilia: Lacertilia: Iguanidae) and the Blue-crowned Motmot Momotus momota (Aves: Coraciiformes: Momotidae). Both are locally abundant and sympatric with some members of most pyrrolizidine alkaloid-containing insect groups (Janzen, 1983; DeVries, 1987). I use these predators to establish a rejection threshold and compare it to amounts reported in ithomiine and danaine butterflies. In addition, I restrict the experiments to one or two days, before long-term toxicity is likely to become important.

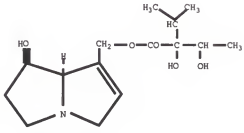
Figure 2-1. Heliotrine, a plant-derived pyrrolizidine alkaloid, and five pyrrolizidine alkaloids found in ithomiine butterflies (Nymphalidae: Ithomiinae; Brown, 1985; Mattocks, 1986). Heliotrine differs from the butterfly alkaloids in stereochemistry and in having an ether group off C 11. Butterfly alkaloids are stereoisomers of one another.



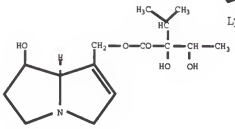
Heliotrine



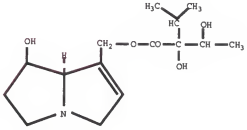
Intermedine



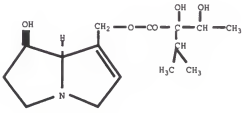
Lycopsamine



Echinatine



Rinderine



Indicine

Materials and Methods

Study Area

This study took place in Monteverde, Puntarenas, Costa Rica, from May 1987 through April 1988. Monteverde lies between 1350 and 1500m elevation in Lower Montane Moist Forest-Wet Forest transition (sensu Holdridge, 1967). The area is home to several insects reported to contain pyrrolizidine alkaloids: ithomiine and danaine butterflies (Lepidoptera: Ithomiinae, Danainae; Haber, 1978; DeVries, 1983), and pyrgomorphid grasshoppers (Orthoptera; Rowell, 1983; Bernays et al., 1977; Ehmke et al., 1989), among others.

Predator Capture and Maintenance

Sceloporus malachiticus

Lizards were captured on fences, houses, and barns with a noose of monofilament line on the end of a pole. Lizard cages were constructed of 1 x 1 cm hardware cloth built to 30 x 30 x 6 cm. A lamp with 15 cm dome shade containing a 60 watt incandescent light was positioned 18 cm above each cage. Lights were controlled by a timer for 12 hours light: 12 hours dark. Lizards were maintained on a diet of mealworms and were caged between one and two weeks on this diet with water before feeding experiments began.

Momotus momota

Birds were mist-netted along roadsides and in forest clearings. Bird cages were constructed of 1.2 x 2.54 cm hardware cloth to a 61 x 61 x 50.8 cm cage. A lamp with 15 cm dome shade containing a 60 watt incandescent light was positioned 18 cm above each cage. Lights were controlled by a timer for 12 hours light: 12 hours dark. Birds were given a diet of egg, carrot, chicken feed, oil, and milk protein supplemented with whole moths and katydids. Birds were caged between one and two weeks on this diet and water before feeding experiments began.

Experimental Design

Rejection of a chemical, or an insect containing it, is a result of both the type of compound and its quantity (Brower, 1984). If a compound is rejected at levels far above those encountered in the environment, it may be unpalatable without ecological relevance. To determine if this might be true for pyrrolizidine alkaloids, a threshold was documented using "up and down" sequential analysis (Brownlee et al., 1953; Dixon, 1965; Dixon and Massey, 1969; Bruce, 1985; Yamanaka et al., 1990). The method has been used to determine emetic thresholds (ED_{50}) of cardenolides for Blue Jays (Cyanocitta cristata bromia; Aves: Corvidae) (Brower et al., 1968; Brower and Fink, 1985). In this method, if an item is eaten, the next item tested contains a

higher dose. If the item is rejected, the next item offered contains a lower dose. After several treatments the concentrations offered should converge around a calculable threshold. Here, I modify the procedure by performing repeated measures over short intervals on single individuals to obtain taste rejection thresholds including replication within individual predators.

The up and down procedure requires data to be normally distributed, dosage levels to be separated by no more than two standard deviations of the (normally distributed transformed) mean (Dixon and Massey, 1969), and the initial dose to be within two dosage levels of the mean (Bruce, 1985). Preliminary experiments were performed to determine these values. The method also includes no control for satiation effects. Preliminary observations as well as pre- and post-trial feedings were used to test satiation (see below). Order effects were tested by Spearman Rank Correlation on data obtained.

Experiments with S. malachiticus

Preliminary experiments

After one week on the laboratory diet, five S. malachiticus were denied food from 1800 to 1000 hours the following day. Next, I fed lizards cabbage looper moths (Lepidoptera: Noctuidae) 10 minutes apart until satiated. All moths were pinched on the thorax to allow crawling but

not flight. After satiation, lizards were then kept an additional week on a maintenance diet. To determine initial dose and between dose interval, these same individuals were next offered moths onto which heliotrine in methanol was applied. I applied the heliotrine solution with 10 ul microcapillary tubes. Predators were offered live moths with 1.0, 10, 100, and 1000 µg heliotrine, beginning with 1.0 µg heliotrine. Control moths held 10 µl methanol.

Feeding experiments

Sceloporus malachiticus were captured and maintained on a maintenance diet for at least 1 week but not more than 2 weeks before feeding experiments began. Individuals tested in preliminary experiments were not included. S. malachiticus were not fed from 1800 the previous night to 1000 the next day. Experiments began at 1000 hours.

All individuals initially ate two methanol-coated moths. These moths had a total 10 µl methanol applied evenly to their thorax, abdomen, and wings with a micropipette. Methanol was allowed to evaporate before moths were offered to predators. Treatment moths were then offered. On these moths, 10 µl methanol was applied that contained varying amounts of heliotrine. As before, moths were offered to S. malachiticus live but incapable of flight. Moths were offered sequentially after methanol had evaporated. Ten minutes passed before offering the next moth. Two methanol

moths, twenty treatment moths, and then two more methanol moths were offered in all.

The amount applied to moths and offered S. malachiticus corresponded to a natural log sequence starting at 500 µg and 0.3 natural log units up or down, for a range of 82 to 1,224 µg. The first treatment moth offered held 500 µg heliotrine. If eaten, the next moth offered contained 672 µg; if the moth with 500 µg heliotrine was rejected, the next moth offered contained 369 µg, and so on. Throughout, I noted any behaviors that might indicate distress.

Experiments with M. momota

Preliminary experiments

Three M. momota maintained one week on laboratory diet were denied food between 1800 and 1000 hours. They were then offered previously frozen cabbage loopers 4 minutes apart until satiated. To determine dosages, after one week on a maintenance diet, these individuals were offered moths onto which heliotrine in methanol was applied using 10 ul microcapillary tubes. Birds were offered moths with 1.0, 10, 100, and 1000 µg heliotrine, beginning with 1.0 µg heliotrine.

Feeding experiments

As suggested by preliminary experiments, the day before observations began M. momota were given five moths onto which the highest dose, 1,224 µg, heliotrine in methanol were

pipetted. Moths were offered after food deprivation from 1800 hours the previous day to 1000 hours the day of presentation. Individuals were then given laboratory diet until 1800 hours. At 1000 hours the following day, birds were first offered two moths onto which 10 µl methanol was applied. A four minute interval elapsed between these and all further moths offered. Two methanol moths, twenty treatment moths, and then two more methanol moths were offered in all.

The amount applied to moths and offered M. momota corresponded to a natural log sequence starting at 500 µg and 0.3 natural log units up or down, for a range of 82 to 1,224 µg. The first treatment moth offered held 500 µg heliotrine. If eaten, the next moth offered contained 672 µg; if the moth with 500 µg heliotrine was rejected, the next moth offered contained 369 µg, and so on. From initial presentation to the end of feeding trials, I noted the incident of distress behaviors.

Results

Sceloporus malachiticus

Preliminary Experiments

Sceloporus malachiticus were capable of eating at least 15 cabbage loopers prior to satiation (16, 18, 18, 20, and 24 moths were eaten). Results from the second set of

preliminary observations were insufficient to determine "up and down" method dosage levels. The interval between test levels was more than twice the standard deviation in all cases (Table 2-1). Therefore, means calculated from these data are unreliable estimates. Still, rejections occurred at 1000 µg and most 100 µg moths were eaten (Figure 2-2). Therefore, the starting level for the following experiment was chosen as 500 µg. The interval between levels was chosen as 0.3 natural log increments because this incorporated 82 to 1,224 µg in ten dosages. This range incorporates most of the range found in ithomiine butterflies in the same area (see Chapter 4).

Sceloporus malachiticus trends did not differ between the first and second days (Table 2-1). A third day of observations, likewise, showed no deviation from the previous day for either species. Therefore, there were no residual inhibitory effects from previous days and I restricted further observations with S. malachiticus to a single day.

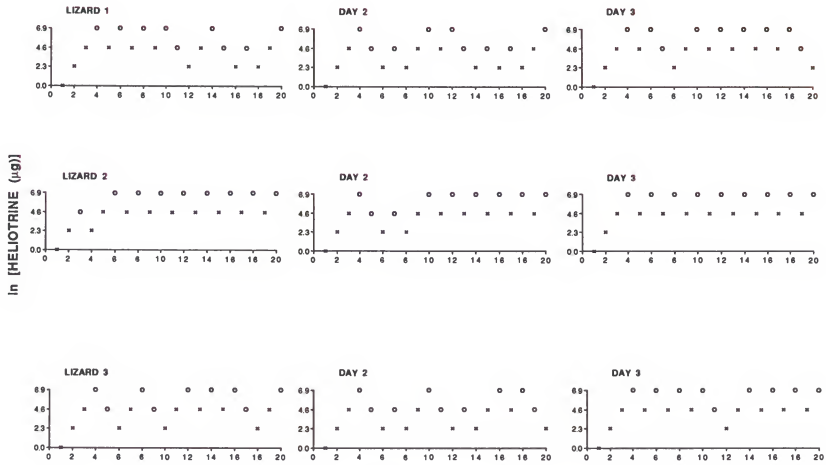
I noted four principal distress behaviors in preliminary tests with S. malachiticus and heliotrine: mouth wiping, tongue flicking, head shaking, and backing away. The first three distress behaviors occurred after eating or releasing a moth. In mouth wiping the cage or forearm was used to repeatedly wipe the mouth. During tongue flicking the tongue slowly protruded into and out of the mouth. While head shaking the head was pivoted side to side rapidly and

Table 2-1. Preliminary dosage trials to determine taste rejection thresholds of a pyrrolizidine alkaloid, heliotrine, applied topically to moths (Lepidoptera: Noctuidae) and offered to two insectivores: Sceloporus malachiticus (Reptilia: family) and Momotus momota (Aves: family).

Species	Individual	Day	Threshold	ln Standard Deviation
S. malchiticus				
	1	1	4.98	0.94
		2	4.47	1.03
		3	5.23	0.75
	2	1	5.49	0.48
		2	5.24	0.75
		3	5.75	1.08
	3	1	4.98	0.94
		2	4.47	1.03
		3	5.49	0.48
M. momota				
	1	2	5.49	0.48
		3	5.75	1.08
	2	2	5.75	1.08
		3	5.75	1.08
	3	2	5.75	1.08
		3	5.75	1.08

Note: All data refer to natural log units to achieve normally distributed data using the "up and down" technique of Dixon and Massey (1969). The sampling interval of 2.3 was larger than twice the standard deviation, and the initial dose of 0 (1 µg) was not within a factor of 2 of the calculated thresholds (see Dixon and Massey, 1969; Bruce, 1985). These results violate conditions of experimental design. Therefore, initial dose and interdose interval could not be determined from these data. However, data suggest that the threshold lies between 100 (4.60 natural log units) and 1000 (6.91) µg heliotrine per moth for both predator species (see also Figures 2-1 and 2-2). Day 1 data for M. momota are not reported because the birds did not discriminate on the first day of exposure.

Figure 2-2. Preliminary "up and down" presentation of heliotrine-painted moths offered Sceloporus malachiticus. Each lizard was offered two methanol moths, twenty heliotrine (in methanol) moths and then two more methanol moths. Only heliotrine-painted moths are represented here. All methanol moths were eaten. If the lizard ate a heliotrine moth (X), the next moth offered held a higher concentration of heliotrine. If the lizard rejected a heliotrine moth (0), the next moth offered contained a lower concentration. S. malachiticus discriminated similarly on all days.


LIZARD 1
DAY 2
DAY 3
LIZARD 2
DAY 2
DAY 3
LIZARD 3
DAY 2
DAY 3
ln [HELIOTRINE (µg)]
PRESENTATION ORDER

violently. Backing away only followed a moth rejection. Upon releasing the moth, lizards would walk backward, increasing the distance between themselves and the rejected moth. Sceloporus malachiticus often combined two or more of these behaviors.

Feeding Experiments

The pyrrolizidine alkaloid heliotrine, applied to moths, was unacceptable to S. malachiticus. They rejected tainted moths, provided heliotrine was present in sufficient quantity. Statistical conditions for threshold determination using the "up and down" method were upheld. All replicates resulted in data that were normally distributed, with dosage intervals less than two natural log increments of the standard deviation and with an initial dose within a factor of 2 of the calculated mean (Table 2-2).

Sceloporus malachiticus showed a taste rejection threshold ranging from 5.59 to 6.26 ln µg, corresponding to 267.7 to 523.2 µg heliotrine (Table 2-2). The mean of means was 5.91, or 383.5 µg (n = 12). Behavioral responses support these calculated thresholds. Incidents of distress were consistently more common above the threshold than below (Table 2-3).

Throughout the trials there were no trends suggesting an order effect, as would be the case for learning or satiation (Figure 2-3; Table 2-4). Satiation may be further disregarded as the last treatment offered was eaten in 41 %

Table 2-2. Taste rejection threshold of the pyrrolizidine alkaloid heliotrine offered to 12 individual S. malachiticus on similarly-sized noctuid moths differing in amount applied.

Individual	Threshold (X +/- s.d.)	$e^{\bar{X}}$	Normality	p
1	5.82 +/- 0.42	337.0 µg	0.201	0.184
2	6.21 +/- 0.33	497.7	0.209	0.175
3	6.26+/- 0.44	523.2	0.246	0.246
4	5.93 +/- 0.20	376.1	0.250	0.132
5	5.66 +/- 0.44	287.1	0.176	0.216
6	6.03 +/- 0.16	415.7	0.227	0.155
7	6.17 +/- 0.61	479.4	0.217	0.166
8	5.59 +/- 0.46	267.7	0.200	0.186
9	5.88 +/- 0.42	357.8	0.154	0.246
10	5.63 +/- 0.35	277.7	0.200	0.186
11	5.89 +/- 0.35	362.6	0.209	0.175
12	6.09 +/- 0.25	441.4	0.224	0.157

Note: Twenty moths were offered sequentially to individual lizards, modifying the "up and down" protocol of Dixon and Massey (1969). If a lizard ate a moth, the next moth offered it contained more heliotrine. If rejected, the moth contained less. This design requires an initial dose within a factor of 2 of the calculated mean, a dose interval within two (transformed) standard deviations, and normally distributed observations. These conditions were satisfied and the related statistics are listed above. The initial dose was 6.21 ln µg heliotrine, corresponding to 500 µg, and the dose interval was 0.3 natural log units. Antilog values for the mean are also reported for convenience. Determinations of normality are based upon natural log increments.

of the cases for lizards (expected is 50 %), and all individuals ate the two methanol moths offered last. Preliminary experiments also showed S. malachiticus readily ate more than 15 untreated moths. Lizards in these subsequent feeding experiments ate 13 or 14 total moths over a slightly longer period.

Momotus momota

Preliminary Experiments

Each of the three M. momota could eat more than 25 moths offered four minutes apart. The experimental protocol for threshold experiments required they eat a maximum of 24, so I stopped offering moths after 25 were eaten. As for S. malachiticus, results from the second set of preliminary observations were insufficient to determine dosage level. They did suggest an effect of initial exposure, however. In addition, distress behaviors were identified.

In the second set of preliminary observations, the three M. momota readily ate moths at 1, 10 and 100 µg heliotrine and five consecutive moths at 1000 µg heliotrine on the first day of exposure (Figure 2-4). The birds showed no signs of aversion, but I ceased the experiment fearing acute toxic effects. The next day, all M. momota showed discrimination between 100 and 1000 µg, never accepting moths with 1000 µg (Figure 2-4). A third day of similar exposure did not greatly alter the threshold or pattern of inhibition (Figure

Table 2-3. S. malachiticus differed in response to heliotrine applied to moths above and below the taste rejection threshold (Table 2-2, Figure 2-2). Data for moths that correspond exactly with the threshold are not counted. Distress behaviors were scored as present or absent and include mouth-wiping, head shaking, and/or toungue-wiping.

	% Trials with Distress	
Individual	Below Threshold	Above Threshold
1	37.5 (n = 8)	58.3 (n = 12)
2	50.0 (6)	83.3 (6)
3	46.1 (13)	100.0 (7)
4	57.1 (14)	83.3 (6)
5	63.6 (11)	77.8 (9)
6	72.7 (11)	88.9 (9)
7	44.4 (9)	72.7 (11)
8	57.1 (7)	69.2 (13)
9	28.6 (7)	61.5 (13)
10	41.7 (12)	75.0 (8)
11	33.3 (6)	85.7 (14)
12	44.4 (9)	66.7 (11)

Note: Incidence of distress is significantly different above and below the calculated threshold, Wilcoxon Matched-pairs Signed-ranks Test, $z = -4.286$, $p = 0.0001$.

Figure 2-3 "Up and down" presentation of heliotrine-painted moths to S. malachiticus. All lizards ate an additional pair of methanol-painted moths before and after the trials represented here. X corresponds to cases where the moth was eaten. O corresponds to cases where the moth was rejected. The resulting taste rejection threshold calculated according to Dixon and Massey (1969) is shown by a dotted line.

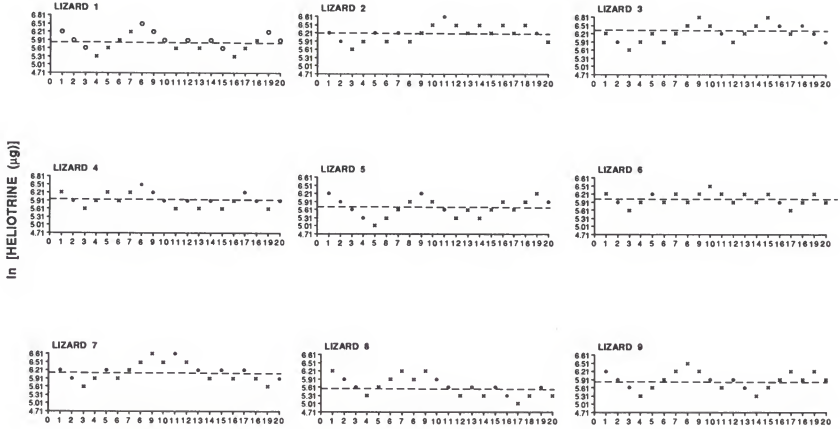

LIZARD 1
LIZARD 2
LIZARD 3
LIZARD 4
LIZARD 5
LIZARD 6
LIZARD 7
LIZARD 8
LIZARD 9
6.81
6.51
6.21
5.91
5.61
5.31
5.01
4.71
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20
ln [HELIOTRINE (µg)]
PRESENTATION ORDER

Table 2-4. Order of heliotrine-painted moth presentation had no effect on response of S. malachiticus and M. momota. Order and dose were compared by Spearman Rank Correlation, where n = 20 in all cases. S. malachiticus demonstrated no tendency to respond differently as the experiment progressed, as shown by the lack of significant correlation between presentation order and dose offered. Two M. momota may have responded, in part, to order of presentation, but in opposite ways, indicated here by a significant positive and negative correlation.

Species	Individual	Statistic	p
S. malachiticus			
	1	0.049`	0.838
	2	0.428	0.062
	3	0.017	0.943
	4	-0.247	0.241
	5	0.088	0.701
	6	0.187	0.430
	7	-0.308	0.187
	8	-0.316	0.175
	9	0.389	0.090
	10	0.253	0.282
	11	0.253	0.282
	12	-0.364	0.115
M. momota	1	0.027	0.911
	2	0.185	0.435
	3	-0.248	0.293
	4	0.053	0.823
	5	-0.046	0.847
	6	0.490	0.028*
	7	-0.688	0.001*
	8	-0.165	0.487

2-4; Table 2-1). Given residual effects on Day 2 but not Day 3, I restricted threshold observations to the second day, after birds had recent prior access to heliotrine on moths. As birds rejected moths with 1000 µg heliotrine but accepted those with 100 µg, the initial dose for subsequent experiments was set at 500 µg, with a dose interval of 0.3 natural log units.

Distress behaviors for M. momota closely resemble those already identified for other bird species in insect unpalatability tests (Bowers, 1981; Schlee, 1986; Ritland, 1991). Bill wiping occurred after picking up a moth with the beak or after eating a moth. Here, the bird would wipe its beak on the perch or side of the cage. Head-shaking also followed touching or eating moths. The head would rapidly vibrate left to right. Feather ruffling followed eating a moth. In this case, the feathers about the head were raised, sometimes extending to feathers on the back. M. momota sometimes exhibited more than one behavior in a single trial.

Feeding Experiments

The pyrrolizidine alkaloid heliotrine, applied to moths, was unacceptable to M. momota. The birds rejected tainted moths, provided heliotrine was present in sufficient quantity and they had consumed heliotrine on moths the day before. Statistical conditions for threshold determination using the "up and down" method were upheld. All replicates resulted in

Figure 2-4. Preliminary "up and down" presentation of heliotrine-painted moths offered Momotus momota. Each bird was offered two methanol moths, twenty heliotrine (in methanol) moths and then two more methanol moths. Only heliotrine-painted moths are represented here. All methanol moths were eaten. If the bird ate a heliotrine moth (X), the next offered was of higher concentration. If the bird rejected a heliotrine moth (0), the next moth offered contained a lower concentration. Day 1 birds ate all moths without regard to heliotrine concentration and the experiment was terminated after eight heliotrine moths were offered. On subsequent days, M. momota was more discriminating.

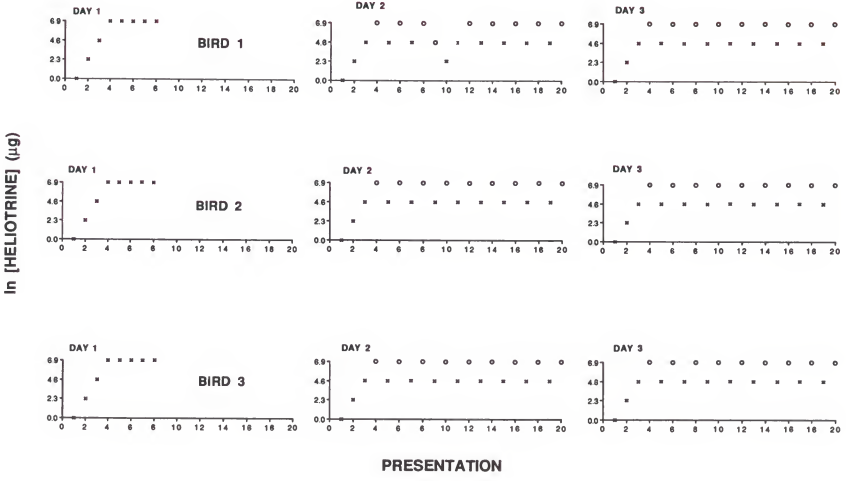

DAY 1
DAY 2
DAY 3
BIRD 1
BIRD 2
BIRD 3
6.9
4.6
2.3
0.0
0 2 4 6 8 10 12 14 16 18 20
ln [HELIOTRINE] (µg)
PRESENTATION

Table 2-5. Taste rejection threshold of the pyrrolizidine alkaloid heliotrine offered to eight M. momota on similarly-sized noctuid moths differing in amount applied. Twenty moths were offered sequentially to individual birds, modifying the "up and down" protocol of Dixon and Massey (1969). Experimental conditions were satisfied and the related statistics are listed below. The initial dose was 6.21 ln µg heliotrine, corresponding to 500 µg, and the dose interval was 0.3 natural log units. Antilog values for the mean are also reported for convenience. Determinations of normality are based upon natural log increments.

Individual	Threshold (X +/- s.d.)	e^X	Normality	p
1	6.59 +/- 0.74	727.8 µg	0.233	0.148
2	6.76 +/- 0.23	862.6	0.244	0.137
3	6.66 +/- 0.80	780.6	0.219	0.164
4	6.42 +/- 0.77	614.0	0.246	0.136
5	6.69 +/- 0.28	807.0	0.225	0.157
6	6.63 +/- 0.17	755.0	0.246	0.136
7	6.45 +/- 0.59	632.7	0.191	0.197
8	6.48 +/- 0.47	652.0	0.247	0.135

Figure 2-5. "Up and down" presentation of heliotrine-painted moths to M. momota. Birds ate five moths containing 6.81 (or 1,224 µg) heliotrine the day before the trials represented here (see text). The next day, all birds ate an additional pair of methanol-painted moths before and after the trials represented here. X corresponds to cases where the moth was eaten. O corresponds to cases where the moth was rejected. The resulting taste rejection threshold calculated according to Dixon and Massey (1969) is shown by a dotted line.

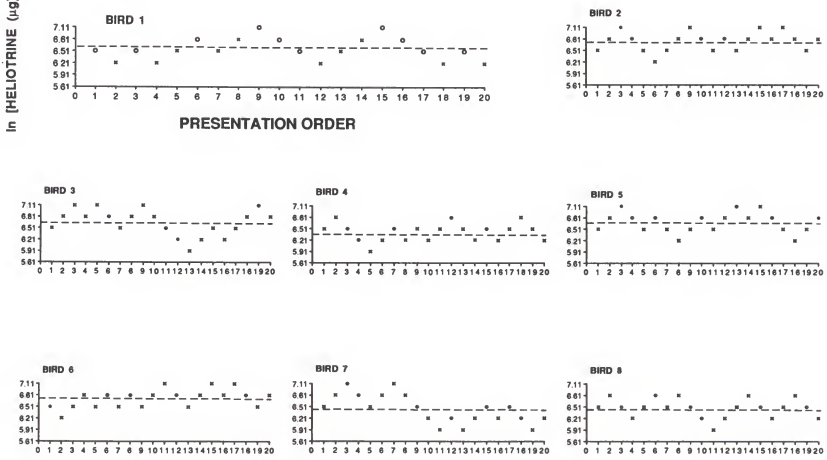

ln [HELIOTRINE (µg)]
BIRD 1
BIRD 2
BIRD 3
BIRD 4
BIRD 5
BIRD 6
BIRD 7
BIRD 8
7.11
6.81
6.51
6.21
5.91
5.61
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20
PRESENTATION ORDER

Table 2-6. M. momota responses to heliotrine applied to moths above and below the taste rejection threshold (Table 2-5, Figure 2-4). Data for moths that correspond exactly with the threshold are not counted. Distress behaviors were scored as present or absent and include beak-wiping, head shaking, and/or feather ruffling.

	% Trials with Distress	
Individual	Below Threshold	Above Threshold
1	15.4 (n = 13)	71.4 (n = 7)
2	25.0 (8)	41.7 (12)
3	20.0 (10)	40.0 (10)
4	0.0 (8)	41.7 (12)
5	30.0 (10)	60.0 (10)
6	33.3 (12)	62.5 (12)
7	22.2 (9)	63.6 (11)
8	16.7 (6)	35.7 (14)

Note: Incidence of distress is significantly different above and below the calculated threshold, Wilcoxon Matched Pairs test, $z = -3.464$, $p = 0.0005$.

data that were normally distributed, with dosage intervals less than two natural log increments of the standard deviation, and with an initial dose within a factor of 2 of the calculated mean (Table 2-5).

The taste rejection threshold for M. momota was between 6.42 and 6.76, or 614.0 to 862.6 µg heliotrine (Table 2-5). This represents a range of just under 250 µg. The threshold mean of means was 6.58, corresponding to 724.1 µg (n = 8). The thresholds were supported by behavioral data, as well. Incidents of distress were more common above the threshold than below (Table 2-6).

Order effects were tested by correlation but no such effects were apparent (Table 2-4). Satiation was also unlikely. The last treatment offered was eaten in 75 % of the cases for birds (expected is 50 %), and all individuals ate the two methanol moths offered last. Furthermore, birds in feeding trials ate between 12 and 14 moths total. Preliminary observations suggest they could eat more than twice this number before satiation occurred.

Comparing S. malachiticus and M. momota

Both predator species rejected heliotrine on moths. Lizards did not require previous exposure to heliotrine in order to begin rejecting moths tainted with it. Instead, S. malachiticus was inhibited from the outset. M. momota, on the other hand, required an initial exposure with some delay

between presentations before the birds began to reject moths by taste alone. The range of taste thresholds, a measure of within species variation in heliotrine taste aversion, was similar in both species, about 250 µg from the most to least sensitive individuals. Species differed in the thresholds themselves, however. The mean taste rejection threshold of M. momota was nearly twice as large as that for S. malachiticus (Mann Whitney U test, $z = -3.703$, $p = 0.0002$).

Discussion

Rejection of heliotrine by S. malachiticus and M. momota supports the hypothesis that pyrrolizidine alkaloids can defend insects. The threshold response shown here also demonstrates that pyrrolizidine alkaloids protect only when present in sufficient quantity. Specifically, the present experiments demonstrate that heliotrine imparts unpalatability to otherwise palatable insects against two vertebrate insectivores. Beyond this, there are three important principles of heliotrine-mediated defense: (1) heliotrine inhibition is probably taste-related and operates on a time scale immediate enough for conditioned avoidance learning, (2) some predators may require previous, possibly repeated ingestion of pyrrolizidine alkaloids before taste rejection begins to operate or continues, and (3) the amount of pyrrolizidine alkaloids necessary to provide protection

varies both among individuals of a species and between species.

Pyrrolizidine Alkaloids as Defense Compounds

Heliotrine made otherwise palatable moths unacceptable to S. malachiticus and M. momota. This is the first evidence that pyrrolizidine alkaloids inhibit insectivory in reptilian and avian insectivores that are likely to encounter pyrrolizidine alkaloid-containing prey. These results and those with spiders (Brown, 1985; Eisner and Eisner, 1991) and rodents (Glendinning et al., 1991) support the more general conclusion that many pyrrolizidine alkaloids inhibit feeding in an array of insectivorous taxa. It suggests, moreover, that pyrrolizidine alkaloids in insects may operate in chemical defense. The addition of S. malachiticus and M. momota to the list of insectivores whose foraging is inhibited by pyrrolizidine alkaloids is also initial evidence that these compounds may be components in the evolution of warning coloration and mimicry. S. malachiticus and M. momota are the first insectivorous species shown to avoid a pyrrolizidine alkaloid that are: sympatric with mimetic pyrrolizidine alkaloid-containing insects, diurnal, and use visual cues in foraging.

Taste Rejection of Heliotrine

Heliotrine moths were rejected by S. malachiticus and M. momota only after placing the moths in their mouths, but

nearly always before eating them. While it is possible that the rejection of moths was olfactory, it is more likely that taste was the principal cue. Alkaloids are bitter compounds and aversion to bitter substances is widespread (Brower, 1984). Therefore, pyrrolizidine alkaloids may offer no adverse effects beyond bitterness and still be effective defense compounds. This may be the case for S. malachiticus with heliotrine. No prior conditioning was necessary during the experiment to induce taste rejection later on. Given their proximity to insects containing pyrrolizidine alkaloids, though, it is likely that these lizards had prior experience. How important prior experience is to S. malachiticus remains untested.

Prior Experience and Conditioned Aversion

Prior experience was necessary for M. momota to begin rejection of heliotrine moths. This must reflect a negative stimulus post-ingestion, as several insects were eaten in less than one half hour with no distress behaviors, including signs of emesis, or other negative physiological effects. Whatever the adverse stimilus, it was sufficiently rapid for a conditioned aversion to form. The following day, birds were rejecting heliotrine moths without eating them. These M. momota probably encountered insects with pyrrolizidine alkaloids before experiments began. The necessity of prior exposure to heliotrine may reflect an inability to generalize from one pyrrolizidine alkaloid to another, or a failure to

generalize pyrrolizidine alkaloid-based aversion from one prey type to another. Alternatively, in M. momota the conditioned aversion may be temporary, requiring intermittent ingestion of pyrrolizidine alkaloids for taste rejection to continue.

Relative Sensitivity to Heliotrine

The threshold measured for heliotrine rejection differed within and between predator species. Both showed similar intraspecific variation, but S. malachiticus showed greater sensitivity to heliotrine than M. momota. The difference may reflect the method of presentation: moths were alive for S. malachiticus but not for M. momota. More likely, these responses reflect the variation already reported in range and laboratory animals (Mattocks, 1986). Brown (1984) reports a threshold for N. clavipes and five purified pyrrolizidine alkaloids found in ithomiines (Figure 2-1) painted onto palatable Biblis sp., which is larger than the moths used here. Above 200 µg, rejection of butterflies by spiders was consistent. Rejection was variable at < 200 µg. Similar amounts of heliotrine and other pyrrolizidine alkaloids fed to ithomiines are capable of making them unpalatable to N. clavipes (Brown, 1985; Masters, 1990). Rodent species are differentially sensitive to monocrotaline, including species that feed on overwintering monarchs (Glendinning et al., 1991). Variation between predators suggests that insectivores span a wide range of pyrrolizidine alkaloid

tolerance, and probably include species entirely insensitive to them.

Ecological Relevance of Pyrrolizidine Alkaloid-based Defense

The question remains, then, are pyrrolizidine alkaloids normally found in levels that confer unpalatability in insects? As it turns out, the mean taste rejection values of 383.5 and 741 µg heliotrine observed are within the ranges of total pyrrolizidine alkaloid content found in many danaine and ithomiine butterflies (Brown, 1984; Brown, 1985; Trigo and Brown, 1990; Chapter 4). This comparison assumes that the varied profile of pyrrolizidine alkaloids found in dananines and ithomiines are similar in taste and toxic action to heliotrine and, further, that most pyrrolizidine alkaloids lie at the surface of the exoskeleton (Brown, 1985). Thus, a tenative examination reveals that 48 of 78 spp. (61 %) examined by Brown (1985) had mean total pyrrolizidine alkaloid contents higher than 383.5 µg, the threshold of heliotrine for S. malachiticus. In total, nearly all ithomiine species (93 %; n = 78 spp.) and all danaine species (n = 4 spp.), including D. plexippus, had maximum pyrrolizidine alkaloid levels exceeding the heliotrine threshold for S. malachiticus. The threshold of 741 µg heliotrine for M. momota shows similar results: 36 % of ithomiine species have greater mean total pyrrolizidine alkaloids (µg) and 74 % show maximum values that exceed 741

µg. All danaines had means higher than this except D. plexippus, which showed a maximum at 731 µg total pyrrolizidine alkaloids.

Such extrapolation further supports the potential defensive function of pyrrolizidine alkaloids in insects. Still, the results of the present experiments are too restrictive to fully explore the ecological importance of pyrrolizidine alkaloid-mediated defense. As shown here, heliotrine, a pyrrolizidine alkaloid, imparts unpalatability to insects if present in sufficient quantity. Even if the pyrrolizidine content of an insect were known, however, both qualitatively and quantitatively, results presented here could not make a positive statement about its unpalatability, except in the special case where heliotrine was the only pharmacologically active compound found. Moreover, heliotrine was applied topically, making results difficult to compare with whole insects.

Better and more direct methods for demonstrating pyrrolizidine alkaloid-mediated defense in insects are needed. Now that I have shown pyrrolizidines are potentially defensive and are taste rejected, Chapter 3 tests predator responses with insects fed pyrrolizidine alkaloids. This is the most direct test that pyrrolizidine alkaloids protect insects that acquire them. In addition, Chapter 4 addresses natural variation in pyrrolizidine alkaloid content in insects, in part by presenting a more realisitic threshold

along with pyrrolizidine alkaloid levels from ithomiine species sympatric with the predators used here.

Summary

Despite their widespread occurrence in insects, the role of pyrrolizidine alkaloids in defense has been the subject of debate. Most pyrrolizidine alkaloid-containing insects appear to be or are demonstrated as unpalatable. Yet, pyrrolizidine alkaloids may be too slow-acting to elicit conditioned aversion or may not naturally occur in sufficient quantity to confer taste avoidance. Pyrrolizidine alkaloids have been shown to inhibit insectivory in spiders (Brown, 1984). I demonstrate inhibitory properties of heliotrine, a pyrrolizidine alkaloid, against two vertebrates insectivores. Heliotrine may be taste rejected by the lizard S. malachiticus without prior experience, but a negative stimulus post ingestion was necessary to elicit taste rejection in the bird M. momota. Thresholds for heliotrine taste rejection differed between the two species, but both were within the range of total pyrrolizidine alkaloid content reported for ithomiine and danaine butterflies.

CHAPTER 3
UNPALATABILITY OF ITHOMIINE BUTTERFLIES
TO VERTEBRATE PREDATORS

Introduction

Over a century ago, Bates (1862) and Müller (1879) devised theories of mimicry to explain nearly identical coloration between butterfly species. Since then, mimicry theory and related studies continue to offer a convincing case of natural selection (Fisher, 1927; Brower, 1988). Ithomiine butterflies (Nymphalidae: Ithomiinae, then referred to as danaoid heliconids) figured prominently in the development of mimicry theory; both Bates and Müller hypothesized ithomiine unpalatability, presumably from a chemical conferring a noxious quality to the butterflies. The idea that ithomiines are protected is predicated on a surprising lack of ithomiine predation by vertebrate predators despite their abundance, bright coloration, slow flight, and diurnal activity (Kaye, 1914). Beak mark frequency (Kaye, 1914; Colenette and Talbot, 1928; Carpenter, 1941; Drummond, 1976; Haber, 1978), wing tenacity (Longstaff, 1908; Kaye, 1914), "peculiar smell" (Bates 1862; Longstaff, 1908, 1912), death-feigning (Gilbert and Ehrlich, 1970; Drummond, 1976); and larval hostplant chemistry (Brower and Brower, 1964; Drummond, 1976; Brown and Vasconcellos-Neto,

1976; Brower, 1985) have been cited as additional support for Bates' and Müller's ithomiine unpalatability hypothesis.

Observations of ithomiine predation, or more appropriate to mimicry theory, rejection of ithomiines by vertebrate predators in nature, are rare. In part, this reflects the inherent difficulty of systematically observing butterfly predation in the field (Dempster, 1984). In addition, if chemical defense and mimicry are effective deterrents, observations of predation should be rare. Most reports of natural predation on ithomiines involve invertebrate predators including spiders (Vasconcellos-Neto and Lewinsohn, 1984; Belt, 1874; Drummond, 1976) and ants (Beebe, 1955; Drummond, 1976) whose role as selective agents for mimetic coloration is questionable. Neither predator relies heavily on visual cues, like coloration, for foraging. Occasionally, there are reports of an insectivorous bird catching, then dropping an ithomiine (Poulton, 1908; Cott, 1940). However, there are also reports of birds eating ithomiines (Haber, 1978), including heavy predation on ithomiines by a population of fawn-breasted tanagers (Pipraeidea melanonota) (Brown and Vasconcellos-Neto, 1976). In an attempt to monitor field predation on ithomiines, Haber (1978) pinned freshly-killed ithomiines to leaves. He found evidence of bird damage in 7 of 58 cases (roughly 12%), compared to 8 of 24 cases (33%) for palatable Anartia fatima (Nymphalidae: Nymphalinae). The sum of these observations is hardly

convincing support for ithomiine unpalatability to vertebrates.

Feeding trials with tame or caged vertebrates provide stronger evidence, although sometimes difficult to interpret. For instance, Belt (1874) fed several wild caught adult ithomiines to a single, tame white-faced monkey (Cebus capucinus) and found the monkey rolled them into a ball and dropped them. Similar observations with single birds (Haber, 1978) or a pair of birds (Chai, 1986) most often show ithomiine rejection. These birds may prefer non-ithomiines, ignore ithomiines, reject them, or eat wild caught adults with some adverse effects, including death in one case (Haber, 1978). Because these studies provide no replication and lack adequate experimental design, conclusions from them remain somewhat tenative. More compelling evidence comes from choice experiments. Given a simultaneous choice of ten butterfly species, Ithomia drymo ranked intermediate in palatability for several individuals of three bird species (Brower and Brower, 1964; Brower, 1984). In addition, I. drymo and two other ithomiines, Hypoleria ocalea and Hypothyris euclea, were largely refused by four temperate bird species that ate all controls (Alcock, 1965, data in Brower, 1984). Such a wide array of choices and the use of temperate predators leaves the most direct test of ithomiine unpalatability undone. Here, I offer a series of simple two-

choice tests to determine ithomiine unpalatability with sympatric, insectivorous vertebrate predators.

In addition to testing the hypothesis of ithomiine unpalatability, it is critical to test the corollary hypothesis of its cause. Perhaps because of mimicry theory, all feeding trials with ithomiines and vertebrate predators have been restricted to adult ithomiines. Larvae were assumed to be unpalatable but were never tested. As a result, investigators advanced the reasonable, yet erroneous, hypothesis that unpalatability in adult ithomiines is caused by solanaceous alkaloids sequestered from larval foodplants (Brower and Brower, 1964; Young, 1972; Brown, 1976; Drummond, 1976; 1981). In fact, solanaceous alkaloids are not reported in adult ithomiines, though numbers assayed are not given (Brown, 1985). Most wild caught adults contain pyrrolizidine alkaloids, however, acquired from several sources in the environment as adults (Brown, 1984; Boppre, 1986; Masters, 1990). Pyrrolizidine alkaloids are avoided by at least two species of spider (Brown, 1985, Eisner and Eisner, 1991). In Chapter 2, I demonstrate that, in sufficient quantity, pyrrolizidine alkaloids are also avoided by vertebrate predators: the Spiny Lizard (Sceloporus malachiticus) and the Blue-crowned Motmot (Momotus momota), both sympatric and potential predators on ithomiines.

Here, I extend studies of ithomiine palatability and chemical defense with vertebrate predators. I examine both

larval and adult stages, including a direct test of pyrrolizidine alkaloid-based unpalatability. In doing so, I find that ithomiine acceptability to vertebrate predators changes with life stage and that the secondary compounds responsible for unpalatability vary. This is only a partial vindication of Bates' and Müller's original hypothesis of ithomiine unpalatability. I show that larval ithomiines are poorly protected and adults must acquire protective compounds post-eclosion to become unpalatable to at least two vertebrate predators.

Materials and Methods

The Predators

Spiny Lizards (Sceloporus malachiticus) and Blue-crowned Motmots (Momotus momota) were captured in Monteverde, Puntarenas Province, Costa Rica between May 1987 and April 1988. Monteverde lies between 1350 and 1500m elevation in Lower Montane Moist Forest-Wet Forest transition (sensu Holdridge, 1967). Lizards were captured on fences, houses, and barns with a noose of monofilament line on the end of a pole. Birds were mist-netted along roadsides and in forest clearings.

Lizard cages were constructed of 1cm x 1cm hardware cloth built into 30 x 30 x 6 cm. Bird cages were constructed of 5 x 10 cm hardware cloth built to a 80 cm cube. A lamp

with 15 cm dome shade containing a 60 watt incandescent light was positioned 18 cm above each cage. Lights were controlled by a timer for 12 hours light: 12 hours dark. Lizards were maintained on a diet of mealworms, birds were given a diet of egg, carrot, chicken feed, oil, and milk protein supplemented with whole moths and katydids. Both species were caged between one and two weeks on these diets before feeding experiments began. Motmots had previously been offered a series of 20 adult noctuid moths with the pyrrolizidine alkaloid heliotrine topically applied (see Chapter 2). Lizards were replaced with newly captured individuals after each experiment. All treatments were performed on the same eight birds.

The Butterflies

All wild caught ithomiines were captured along the Rio Guacimal within the Monteverde community. Twenty-one tiger-stripe or gold translucent (sensu Haber, 1978) ithomiine species were captured there during the course of this study, from April 1987 to May 1988. There were also several species of clear-winged ithomiines that were not studied. Potential non-ithomiine mimics (any butterfly with similar coloring) in the area were restricted to Lycorea cleobaea (Nymphalidae: Danainae), which were rare (see Chapter 4).

The tiger-stripe ithomiine butterfly Pteronymia fulvescens was reared on Solanum brenesii (Solanaceae).

Wild-caught females were placed in flight cages with cuttings and readily oviposited on leaves. Leaves with eggs were placed in plastic bags. Upon hatching, larvae were reared six to a bag until the final instar, when larvae were isolated from one another. Leaves were replaced daily and the amount of leaves were kept at a level producing a small amount of condensation on the inside of the bag. The temperature of the rearing room was not measured, but the room was well-ventilated and ambient temperatures outside fluctuated around 23° C, ranging from 22 to 24°C during the time of rearings. Larvae have a black head capsule, a dark brown body color with two yellow dorsolateral stripes running their entire length, a bright yellow area behind the head, and a black anal plate. They normally feed on the underside of leaves. I believe their color to be cryptic at a distance but it may be aposematic to a predator at close proximity. Pupation occurred on the bag. Pupae were removed and placed in hanging mesh cages. Adults were misted daily with water.

Some adults were fed known volumes of solution with a calibrated 100 µl microcapillary tube on a platform. These butterflies were fed 10 µl per day of a 30 % sucrose solution or a similar sucrose solution with 10 µg per µl heliotrine, a pyrrolizidine alkaloid, for four days. I then starved these butterflies for one day before either offering them to lizards, or freezing them to use up to six months later with birds. Heliotrine does not occur in ithomiines but is

readily available to them from boraginaceous plants. I used it here because it was readily available in purified form (Sigma Chemical Co.) and closely resembles the structures of pyrrolizidine alkaloids found in ithomiines (Figure 2-1).

I used mealworms as controls with ithomiine larvae and adult Anartia fatima (Nymphalidae: Nymphalinae) for comparisons with ithomiine adults. Anartia spp. have proven consistently palatable to vertebrates in other feeding experiments (e.g., Brower and Brower, 1964; Boyden, 1976). There are many observations of avian and reptilian insectivores attacking A. fatima in the field and A. fatima is common in the diets of insectivores inhabiting second-growth areas, as seen from examining stomach contents (see review in Silberglied et al., 1979).

Feeding Experiments

I conducted palatability experiments that compared the acceptability of larval and adult ithomiines with palatable controls. In these experiments, lizards or birds were starved from 1800 the night before to 0900 the day of feeding trials. All subjects were given an initial palatable mealworm or A. fatima. Next, a pair consisting of a single control and a single ithomiine were offered sequentially. To eliminate any bias associated with the order of presentation, a coin was tossed so that control and treatement order were

determined randomly. Insects were offered live to lizards. Mealworms and larvae were free to roam the cage bottom but were removed and replaced if they began to climb. Adult butterflies were pinched on the thorax to allow walking but not flight. Insects were offered dead to birds; these insects had been previously frozen.

Lizards and birds were given one minute to react before the item was removed from the cage. Reactions were scored as 0 = not touched (ignored but seen), 1 = grabbed and released or pecked, 2= partially eaten, or 3 = entirely eaten. During this time, I also recorded handling time as the amount of time elapsed between touching the item and it being either rejected or eaten. Distress behaviors were also noted. Five minutes elapsed before the next item was offered. After the final presentation for the day, a palatable insect, corresponding to the control, was offered to test for possible satiation. For lizards, 12 insects were offered (including 5 pairs) each day for two consecutive days. For birds, 22 insects (including 10 pairs) were offered each day. One day was allowed to elapse between successive experiments.

I tested the palatability of P. fulvescens larvae, newly emerged adults, sucrose-fed adults, heliotrine-fed adults (a pyrrolizidine alkaloid), and field-captured adults. Larvae (5th instar) were presented alternatively with mealworms, and newly emerged adult P. fulvescens were presented with A. fatima as a control. To test the effect of pyrrolizidine

alkaloids on ithomiine palatability, heliotrine-fed adults were offered in pairs with sucrose-fed ones. The two P. fulvescens groups were the same in every way except for their differential exposure to heliotrine. Therefore, any difference in the time or outcome of the encounter must be due solely to the presence of this pyrrolizidine alkaloid. Finally, field-captured adults were offered with a unique control group: newly-emerged adult P. fulvescens offered only water.

Conclusions from these experiments are drawn from four lines of related evidence with P. fulvescens and appropriate controls: the items that were eaten, how predators approached prey, incidence of distress behaviors, and handling time. Refusing to eat an insect is the clearest evidence of unpalatability. However, if the predators' responses are examined in greater detail they may suggest that, while eating items, the predator is more hesitant in some cases than others. Response scores quantify the outcome of a presentation so that several replicates summarize a subject's choices. Distress behaviors are interpreted as indicating a disagreeable feature to the prey. Handling times for items eaten might be expected to increase with unpalatable insects if the predator is hesitant to eat it. If an item is rejected, however, the time spent with it might be long or short, depending upon the ease of tasting or learned avoidance. Here, I include only handling times for

items eventually eaten. Finally, such handling times might lengthen with exposure, producing a positive correlation between presentation order and handling time.

I compare the number of items eaten for control and treatment for each experiment using a Wilcoxon Matched-Pairs Signed-Rank Test (Siegel, 1956). I compare mean response score, number of distress behaviors, and mean handling times (including items eaten only) for each experiment using this same test. When possible (when greater than 7 items were eaten in both groups), I compare handling times and presentation order using a Spearman Ranks Correlation Test (Siegel, 1956).

Results

Mealworms vs. P. fulvescens Larvae.

While neither S. malachiticus nor M. momota found P. fulvescens larvae entirely palatable (number eaten for control vs. treatment were statistically different, $p < 0.05$) the majority of P. fulvescens larvae were eventually eaten.

Sceloporus malachiticus

For lizards, 91% of all P. fulvescens were eaten, compared to 99% of all mealworms (Figure 3-1). In spite of the high number eaten, other evidence suggests larval unpalatability. Lizard response scores to larvae and mealworms were also significantly different (see Table 3-1),

indicating that S. malachiticus more often chose to damage, nudge or ignore P.fulvescens larvae than mealworms, which were readily eaten in all but one case (Figure 3-1). In contrast, 25% of S. malachiticus consumed all 10 P. fulvescens larvae offered, 62.5% rejected a single P. fulvescens larva, and the remaining lizard (representing 12.5%) rejected two larvae after nudging them. In half of the lizards, presentation order influenced the response of S. malachiticus to P. fulvescens larvae so that initial larvae were eaten more quickly (Table 3-1). In addition, S. malachiticus exhibited mouth wiping and tongue flicking immediately after eating some P. fulvescens larvae but not mealworms. Mouth wiping consisted of either wiping the mouth along the cage or with the front feet. Tongue flicking was a slow extension and retraction of the tongue several times in succession. Mouth wiping and tongue flicking sometimes occurred together. These behaviors occurred in six of the eight S. malachiticus with some P. fulvescens larvae presentations; a total of 16% of the cases.

Momotus momota

M. momota found P. fulvescens larvae more unpalatable than did S. malachiticus (Mann-Whitney U test for larval categories only, $p < 0.05$). Still, 75% of the larvae were eventually eaten (Figure 3-2). While the number of P. fulvescens larvae eaten per bird ranged from half to all ten larvae, the mean number of larvae rejected was 2.5 (S.D. =

Figure 3-1. Comparative palatability of Pteronymia fulvescens (Nymphalidae: Ithomiinae) to the Spiny Lizard (Sceloporus malachiticus). Bars represent the number of items eaten in four experiments comparing: mealworms and larvae, Anartia fatima (Nymphalidae: Nymphalinae) and P. fulvescens reared on S. brenesii, similar butterflies fed sucrose or sucrose with a pyrrolizidine alkaloid (PA, in this case heliotrine), and reared adults compared to P. fulvescens adults from the forest (wild). The groups are compared using the Wilcoxon Matched-Pairs Signed Ranks Test at $p < 0.05$, $n = 8$ predators.

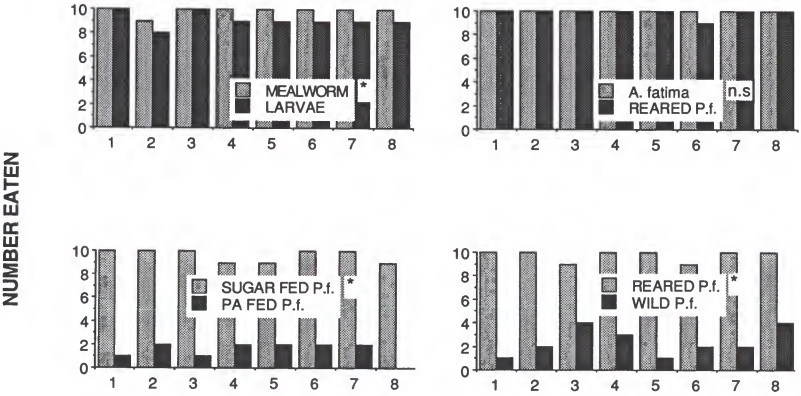

NUMBER EATEN
MEALWORM
LARVAE
*
A. fatima
REARED P.f.
n.s
SUGAR FED P.f.
PA FED P.f.
*
REARED P.f.
WILD P.f.
*
LIZARD

Table 3-1. Differential responses by Sceloporus malachiticus to mealworms ("M" below) and larval P. fulvescens. Mean response score is calculated from ten paired trials per lizard where 0 = item not touched, 1 = grabbed and released, 2 = macerated/partially eaten, and 3 = entirely eaten. Therefore, a lower score indicates lower acceptability. Number of distress behaviors indicates how many trials included mouth wiping or head shaking (for description see text). Handling times reported are means representing the time interval between contact and rejection or ingestion. Scores, incidence of distress, and handling times are compared for mealworms and larvae by Wilcoxon Matched-Pairs Signed Ranks test on the means reported in this table with p values listed below. Order effects on handling times for items eventually eaten were tested for significance by Spearman Rank Correlation. The correlation coefficient (R) is reported and significance ($p < 0.05$) is represented by asterisk (*).

	Mean Response Score		No. Distress Encounters		Mean Handling Time (sec.)		R, Order Effects	
Lizard	M	Larvae	M	Larvae	M	Larvae	M	Larvae
1	3.0	3.0	0	1	5.2	9.4	0.55	0.52
2	2.9	2.6	0	4	3.9	9.6	0.08	0.57
3	3.0	3.0	0	0	4.7	15.2	0.27	0.74*
4	3.0	2.7	0	2	6.2	12.7	0.15	0.70*
5	3.0	2.7	0	1	3.3	13.1	-0.32	0.64*
6	3.0	2.9	0	0	5.7	8.7	-0.19	0.58
7	3.0	2.8	0	2	5.9	13.3	0.21	0.81*
8	3.0	2.7	0	3	3.7	13.4	-0.48	0.40
Mean	3.0	2.8	0	1.6	4.8	11.9		
S.D.	0.04	0.15	0	1.4	1.1	2.3		
p value	0.05		0.05		0.01			

1.8; n = 8 birds), compared to 0.25 mealworms (S.D. = 0.46, Figure 3-2). As for S. malachiticus, motmot response scores were consistently lower for P fulvescens larvae than mealworms, indicating a greater tendency to avoid them (Table 3-2). In addition, the birds exhibited beak wiping, feather ruffling, and head shaking with P. fulvescens larvae but not with mealworms. Birds would wipe their beak on the perch, cage, or use one of their feet in an effort to clear away any remaining butterfly material around the mouth. Feathers were ruffled first on the head and eventually the rest of the body and were generally accompanied by shaking the head and body at the same time. At times, the feathers were raised and the head shook to the right and left. All but one bird showed at least one of these behaviors while handling P.fulvescens larvae but no birds showed any distress with mealworms. Beak wiping, feather ruffling or head shaking was witnessed in 22% of all encounters between M. momota and P. fulvescens larvae. Handling times for P. fulvescens larvae eventually eaten were more than twice as long as for mealworms consumed. There is also evidence that presentation order affected handling time for five of the birds (Table 3-2). This indicates they were increasingly wary of eating P. fulvescens larvae with experience, but this did not happen with mealworms.

Figure 3-2. Comparative palatability of Pteronymia fulvescens (Nymphalidae: Ithomiinae) to Blue-crowned Motmots (Momotus momota). Bars represent the number of items eaten in four experiments comparing: mealworms and larvae, Anartia fatima (Nymphalidae: Nymphalinae) and P. fulvescens reared on S. brenesii, similar butterflies fed sucrose or sucrose with a pyrrolizidine alkaloid (PA, in this case heliotrine), and reared adults compared to P. fulvescens adults from the forest (wild). The groups are compared using the Wilcoxon Matched-Pairs Signed Ranks Test at $p < 0.05$, $n = 8$ predators.

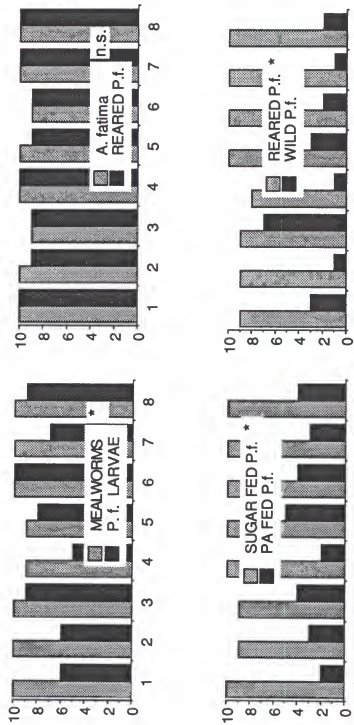

NUMBER EATEN
MEALWORMS
P. f. LARVAE
*
A.fatima
REARED P.f.
n.s.
SUGAR FED P.f.
PA FED P.f.
*
REARED P.f.
WILD P.f.
*
BIRD

Table 3-2. Differential responses by M. momota to mealworms ("M" below) and larval P. fulvescens. Mean response score was calculated from ten paired trials per bird where 0 = item not touched, 1 = pecked or grabbed and released, 2 = macerated or partially eaten, and 3 = entirely eaten. Therefore, a lower score indicates lower acceptability. Number of distress behaviors indicates how many trials included, feather ruffling or head shaking (for description, see text). Handling times reported are means representing the time interval between contact and rejection or ingestion. Scores, incidence of distress, and handling times are compared for mealworms and larvae by Wilcoxon Matched-Pairs Signed Ranks test on the means reported in this table with p values listed below. Order effects on handling times for items eventually eaten were tested for significance by Spearman Rank Correlation. The correlation coefficient (R) is reported and significance ($p < 0.05$) is represented by asterisk (*).

	Mean Response Score		No. Distress Encounters		Mean Handling Time (sec.)		R, Order Effects	
Bird	M	Larvae	M	Larvae	M	Larvae	M	Larvae
1	3.0	2.4	0	2	5.1	21.1	0.12	0.77*
2	3.0	2.4	0	3	3.2	11.0	-0.24	0.81*
3	3.0	2.9	0	2	4.2	13.8	-0.69*	0.84*
4	2.7	2.1	0	2	5.7	11.9	0.01	0.47
5	2.8	2.6	0	3	6.0	13.3	-0.32	0.74*
6	3.0	3.0	0	0	2.9	4.7	-0.19	0.12
7	3.0	2.2	0	4	4.9	15.8	0.21	0.74*
8	3.0	2.7	0	2	7.6	14.3	-0.48	0.60
Mean	2.9	2.5	0	2.2	5.0	13.2		
S.D.	0.04	0.32	0	1.2	1.5	4.6		
p value	0.05		0.01		0.05			

A. fatima and Reared P. fulvescens Adults

Pteronymia fulvescens reared on Solanum brenesii and offered only water as adults were palatable to both S. malachiticus and M. momota. There was no significant difference between the way these predators handled A. fatima and P. fulvescens.

Sceloporus malachiticus

Lizards ate all A. fatima and all but one P. fulvescens, which was ignored in the initial trial for one lizard (Figure 3-1). In this case, response scores do not provide additional information (Table 3-3), as all scored 3.0 (corresponding to an item being eaten). The obvious exception was the single P. fulvescens that was ignored, yielding a mean response score of 2.7 (Table 3-3) for one S. malachiticus individual. The distress behaviors previously mentioned were not observed for any lizards in these trials. Handling times were, likewise, similar for both butterfly species and did not change with presentation order (Table 3-3).

Momotus momota

Motmots, likewise, treated A. fatima and P. fulvescens similarly. Nearly all butterflies were eaten in this experiment: 98% of A. fatima and 95% of P. fulvescens (Figure 3-2). Two M. momota ignored the first A. fatima

Table 3-3. Similar responses by S. malachiticus to A. fatima ("A. f." below) and P. fulvescens ("P. f.") reared on S. brenesii and offered only water as adults. Mean response score is calculated from ten paired trials per lizard where 0 = item not touched, 1 = grabbed and released, 2 = macerated/partially eaten, and 3 = entirely eaten. Therefore, a lower score indicates lower acceptability. Number of distress behaviors indicates how many trials included mouth wiping or head shaking (for description, see text). Handling times reported are means representing the time interval between contact and rejection or ingestion. Scores, incidence of distress, and handling times are compared for the two butterfly species offered by Wilcoxon Matched-Pairs Signed Ranks test on the means reported in this table with p values listed below. Order effects on handling times for items were tested for significance by Spearman Rank Correlation. The correlation coefficient (R) is reported and a significance difference ($p < 0.05$) is represented by asterisk (*).

	Mean Response Score		No. Distress Encounters		Handling Time (sec.)		R, Order Effects	
Lizard	A.f.	P.f.	A.f.	P.f.	A.f.	P.f.	A.f.	P.f.
1	3.0	3.0	0	0	8.6	11.9	0.12	0.30
2	3.0	3.0	0	0	10.3	9.7	-0.41	0.09
3	3.0	3.0	0	0	7.5	9.2	0.21	-0.36
4	3.0	3.0	0	0	6.9	6.7	-0.35	-0.60
5	3.0	3.0	0	0	7.2	7.1	0.12	0.09
6	3.0	2.7	0	0	9.1	6.2	0.51	0.32
7	3.0	3.0	0	0	8.1	9.8	0.21	0.02
8	3.0	3.0	0	0	7.7	7.9	-0.11	-0.26
Mean	3.0	3.0	0	0	8.2	8.7		
S.D.	0.00	0.11	0	0	1.1	1.9		
p value	n.s.		n.s.		n.s.			

Table 3-4. Similar responses by M. momota to wild caught A. fatima ("A. f." below) and P. fulvescens ("P. f.") reared on S. brenesii and offered only water as adults. Mean response score is calculated from ten paired trials per bird where 0 = item not touched, 1 = grabbed and released, 2 = macerated/partially eaten, and 3 = entirely eaten. Therefore, a lower score indicates lower acceptability. Number of distress behaviors indicates how many trials included bill wiping or head shaking (for description see text). Handling times reported are means representing the time interval between contact and rejection or ingestion. Scores, incidence of distress, and handling times are compared for the two butterfly species offered by Wilcoxon Matched-Pairs Signed Ranks test on the means reported in this table with p values listed below. Order effects on handling times for items were tested for significance by Spearman Rank Correlation. The correlation coefficient (R) is reported and a significance difference ($p < 0.05$) is represented by asterisk (*).

	Mean Response Score		No. Distress Encounters		Handling Time (sec.)		R, Order Effects	
Bird	A.f.	P.f.	A.f.	P.f.	A.f.	P.f.	A.f.	P.f.
1	3.0	3.0	0	0	2.8	2.6	0.32	0.08
2	3.0	3.0	0	0	3.1	5.4	0.22	-0.13
3	2.7	2.7	0	0	6.3	4.1	0.40	-0.55
4	3.0	3.0	0	0	6.2	5.7	-0.08	-0.22
5	3.0	2.8	0	0	3.7	3.9	-0.43	-0.72*
6	2.8	2.7	0	0	4.4	3.2	0.42	0.27
7	3.0	3.0	0	0	5.9	6.0	-0.45	0.20
8	3.0	3.0	0	0	4.9	2.9	-0.61	-0.52
Mean	2.9	2.9	0	0	4.7	4.2		
S.D.	0.12	0.14	0	0	1.4	1.3		
p value	n.s.		n.s.		n.s.			

offered but regularly accepted subsequent ones. In one case, this also happened with P. fulvescens. In the other three cases, birds picked up the first P. fulvescens offered and, without macerating, threw it out of the dish (scored as a peck). Subsequent P. fulvescens, however, were eaten without hesitation. Handling times were similar for the two butterfly species and presentation order did not increase total time spent on an item. In one case, a negative correlation was observed (Table 3-4), indicating the bird showed an increased likelihood of eating the reared P. fulvescens with increased exposure to these butterflies. As with lizards, distress behaviors in birds were not observed in any trials.

Sugar-fed vs. Pyrrolizidine Alkaloid-fed P. fulvescens Adults

The pyrrolizidine alkaloid, heliotrine, when taken in a sucrose solution by adult P. fulvescens, conferred unpalatability. In the majority of cases, S. malachiticus and M. momota rejected heliotrine-fed P. fulvescens but readily consumed P. fulvescens offered a sucrose solution.

Sceloporus malachiticus

Sceloporus malachiticus found heliotrine-fed adults less acceptable than similar adults fed sucrose solution alone (Figure 3-1). P. fulvescens adults fed sucrose only were relatively palatable, eaten in 96% of the cases compared with only 15% of P. fulvescens given the pyrrolizidine alkaloid

Table 3-5. Differential responses by S. malachiticus to P. fulvescens fed 40 µl 20% sucrose solution ("SUGAR" below) or a similar solution with 10 µg/µl heliotrine (a pyrrolizidine alkaloid), hence 400 µg total ("PA" below). Mean response score is calculated from ten paired trials per lizard where 0 = item not touched, 1 = grabbed and released, 2 = macerated/partially eaten, and 3 = entirely eaten. Therefore, a lower score indicates lower acceptability. Number of distress behaviors indicates how many trials included bill wiping or head shaking (for description see text) after making contact with a butterfly. Handling times reported are means representing the time interval between contact and ingestion. Handling times for items not eaten are not included. Scores, incidence of distress, and handling times are compared for control and treatments groups by Wilcoxon Matched-Pairs Signed Ranks test on the means reported in this table with p values listed below.

	Mean Response Score		No. Distress Encounters		Handling Time (sec.)	
Lizard	SUGAR	PA	SUGAR	PA	SUGAR	PA
1	3.0	1.3	0	3	5.5	14.0
2	3.0	1.8	0	4	3.7	17.9
3	3.0	1.4	0	4	4.6	19.2
4	2.7	1.9	0	5	6.9	13.8
5	2.7	1.7	0	3	7.0	12.1
6	3.0	1.7	0	6	5.1	10.7
7	3.0	1.5	1	8	6.4	9.8
8	2.7	1.5	0	4	4.8	18.6
Mean	2.9	1.6	0.1	4.6	5.5	14.5
S.D.	0.16	0.21	0.3	1.7	1.2	3.6
p value	0.01		0.01		0.01	

heliotrine. Response scores were also lower for pyrrolizidine alkaloid-exposed P. fulvescens, indicating that pyrrolizidine-alkaloid butterflies were largely ignored or nudged (Table 3-5). In this instance, such butterflies were most often nudged. Remember, lizards had no visual cues, as both treatment and control were similar in appearance. S. malachiticus showed more signs of distress after eating butterflies from the pyrrolizidine alkaloid group than the butterflies fed sucrose alone. All lizards showed distress with at least three alkaloid-fed butterflies, with an overall mean of 46%, but showed distress behaviors in just 1% of cases with sucrose-fed P. fulvescens.

Momotus momota

Pyrrolizidine alkaloid exposure also made most P. fulvescens unpalatable to M. momota (Figure 3-2), though not as often as with S. malachiticus (Mann Whitney U Test, $p < 0.05$). As a group, motmots rejected just two sugar-fed butterflies (2.5%) compared to 53 heliotrine-fed butterflies (66%). By contrast, S. malachiticus rejected only one more sugar-fed butterfly (3.8%) than M. momota but rejected a total of 65 (85%) pyrrolizidine alkaloid-fed butterflies. Further evidence of pyrrolizidine alkaloid-based unpalatability to motmots comes from the significant decrease in response score for heliotrine-fed P. fulvescens (Table 3-6). Distress behaviors were consistently found in encounters with alkaloid-fed butterflies, occurring in 31% of all cases

Table 3-6. Differential responses by M. momota to P. fulvescens fed 40 µl 20% sucrose solution ("SUGAR" below) or a similar solution with 10 µg/µl heliotrine (a pyrrolizidine alkaloid), hence 400 µg total ("PA" below). Mean response score is calculated from ten paired trials per bird where 0 = item not touched, 1 = pecked or grabbed and released, 2 = macerated/partially eaten, and 3 = entirely eaten. Therefore, a lower score indicates lower acceptability. Number of distress behaviors indicates how many trials included bill wiping or head shaking (for description see text) after making contact with a butterfly. Handling times reported are means representing the time interval between contact and ingestion. Handling times for items not eaten are not included. Response scores, incidence of distress, and handling times are compared for control and treatments groups by Wilcoxon Matched-Pairs Signed Ranks test on the means reported in this table, with p values listed below.

	Mean Response Score		No. Distress Encounters		Mean Handling Time (sec.)	
Bird	SUGAR	PA	SUGAR	PA	SUGAR	PA
1	3.0	2.1	0	3	3.4	10.7
2	2.8	2.1	0	2	6.3	16.0
3	2.8	1.8	1	4	5.8	12.7
4	3.0	1.6	0	5	3.7	18.5
5	3.0	2.5	0	2	5.4	12.1
6	3.0	2.2	0	4	5.7	14.4
7	3.0	2.6	1	2	4.9	11.1
8	3.0	2.1	0	3	3.1	20.0
Mean	3.0	2.1	0.3	3.1	4.8	14.4
S.D.	0.09	0.33	0.5	1.1	1.2	3.4
p value	0.01		0.01		0.01	

compared to 3% of cases for birds responding to sucrose-fed individuals. In addition, handling times were three times longer for butterflies exposed to alkaloids (Table 3-6).

Reared vs. Wild P. fulvescens Adults

Similar patterns of unpalatability emerge for P. fulvescens captured in the forest. Compared to adults reared on S. brenesii and offered only water, most forest individuals were unpalatable to both predator species. S. malachiticus and M. momota found reared P. fulvescens palatable and to the same degree: 95% eaten and 98% eaten, respectively. Both predator species rejected 76% of the forest P. fulvescens offered.

Sceloporus malachiticus

S. malachiticus demonstrated lower mean response scores with forest butterflies than reared ones (Table 3-7). While two reared P. fulvescens were ignored, the rest were readily eaten. Forest butterflies, however, were commonly nudged or macerated before being rejected. Additionally, forest individuals elicited mouth wiping and head shaking in all lizards: overall, in some 36% of the trials. This happened in only one case for reared P. fulvescens, corresponding to 1% of those trials. Handling times were, likewise, more than double for forest individuals compared to reared butterflies.

Table 3-7. Differential responses by S. malachiticus to P. fulvescens adults reared on S. brenesii offered only water as an adult (LAB) and P. fulvescens adults from the forest (WILD). Mean response score is calculated from ten paired trials per lizard where 0 = item not touched, 1 = grabbed and released, 2 = macerated/partially eaten, and 3 = entirely eaten. Therefore, a lower score indicates lower acceptability. Number of distress behaviors indicates how many trials included bill wiping or head shaking (for description see text) after making contact with a butterfly. Handling times reported are means representing the time interval between contact and ingestion. Handling times for items not eaten are not included. Scores, incidence of distress, and handling times are compared for the two butterfly groups by Wilcoxon Matched-Pairs Signed Ranks test on the means reported in this table with p values listed below.

	Mean Response Score		No. Distress Encounters		Mean Handling Time (sec.)	
Lizard	LAB	WILD	LAB	WILD	LAB	WILD
1	3.0	1.7	0	3	4.7	10.2
2	3.0	2.0	0	4	5.9	18.0
3	2.7	2.1	0	6	6.3	12.7
4	3.0	2.1	0	5	4.9	14.0
5	3.0	1.5	0	3	3.2	12.3
6	2.7	1.7	0	2	7.0	17.1
7	3.0	1.4	1	3	5.2	13.6
8	3.0	2.1	0	3	4.4	12.9
Mean	2.9	1.8	0.1	3.6	4.8	13.8
S.D.	0.05	0.10	0.3	1.3	1.8	2.6
p value	0.01		0.01		0.01	

Table 3-8. Responses of M. momota to P. fulvescens adults reared on S. brenesii offered only water as an adult (LAB) and P. fulvescens adults from the forest (WILD). Mean response score is calculated from ten paired trials per bird where 0 = item not touched, 1 = grabbed and released, 2 = macerated/partially eaten, and 3 = entirely eaten. Therefore, a lower score indicates lower acceptability. Number of distress behaviors indicates how many trials included bill wiping or head shaking (for description see text) after making contact with a butterfly. Handling times reported are means representing the time interval between contact and ingestion. Handling times for items not eaten are not included. Scores, incidence of distress, and handling times are compared for the two butterfly groups by Wilcoxon Matched-Pairs Signed Ranks test on the means reported in this table with p values listed below.

	Mean Response Score		No. Distress Encounters		Mean Handling Time (sec.)	
Bird	LAB	WILD	LAB	WILD	LAB	WILD
1	2.8	2.1	1	5	3.9	7.4
2	2.8	1.9	0	3	4.5	15.6
3	2.6	2.7	0	2	2.8	11.2
4	2.4	1.8	1	3	5.9	18.1
5	3.0	2.3	0	1	6.5	10.7
6	3.0	2.1	0	2	4.0	13.5
7	3.0	1.3	1	4	7.2	18.9
8	3.0	1.9	0	2	3.6	17.7
Mean	2.8	2.0	0.4	2.8	4.8	14.1
S.D.	0.05	0.40	0.5	1.3	1.5	4.1
p value	0.01		0.01		0.01	

Momotus momota

As before, unpalatability is also demonstrated by statistically different response scores for motmots (Table 3-8). Here, though, response scores are more similar for forest butterflies due to an increased incidence of macerating the butterflies before rejecting them. Ignoring and pecking accounted for all of the reared butterflies rejected, but in just 13% of the cases in which forest butterflies were rejected. Distress behaviors were more common when birds were exposed to forest butterflies, occurring in 28% of the cases, compared to just 4% of reared butterflies. In addition, handling times for items consumed were, on average, nearly three times greater for forest butterflies.

Overview of Results

The palatability of the ithomiine P. fulvescens varied both with life stage and exposure to pyrrolizidine alkaloids (Tables 9 and 10). Larvae, adults exposed to pyrrolizidine alkaloids, and adults captured in the field were unpalatable to varying degrees, while reared adults offered water and sugar were nearly uniformly palatable.

Sceloporus malachiticus

Lizards preferred P. fulvescens in the following order: reared or sugar-fed adults, larvae, wild-caught adults, and adults fed heliotrine. Although all were statistically

similar to controls, larvae differed from reared and sugar-fed adults in causing a greater incidence of distress behaviors and longer handling times (Table 3-9). Wild-caught adults and those fed heliotrine were much more likely to be rejected than other treatment groups: larvae, reared and sugar-fed adults suffered over 90% predation, while P. fulvescens fed heliotrine were eaten in just 15% of cases. Forest butterflies were eaten more readily, but not greatly. Forest butterflies were 9% more likely to be eaten than heliotrine fed adults (24 vs. 15%). In addition, forest butterflies elicited 12% fewer ignores/nudges and 10% fewer distress behaviors than helitorine-fed ithomiines. Despite this, handling times and response scores were similar.

Momotus momota

Reared and sugar-fed adult P. fulvescens were readily eaten by M. momota, unlike larvae, heliotrine-fed adults, and adults caught in the forest (Table 3-10). Reared and sugar-fed adults were nearly universally palatable. Larvae, however, were rejected in 25% of the encounters. Other signs of unpalatability were greater for larvae, as well. Larvae were more likely to be ignored/pecked, elicit distress behaviors, longer handling times, and lower response scores. Still more unlikely to be eaten were heliotrine-fed or wild-caught adults. These two groups also had the highest incidence of ignored/pecked encounters, encounters with distress behaviors, longest handling times, and lowest

Table 3-9. Comparison of ithomiine P. fulvescens treatments by the Spiny Lizard (Sceloporus malachiticus). Treatments are: larvae, adults reared on S. brenesii (REARED), similar adults fed sucrose (SUGAR) or sucrose plus the pyrrolizidine alkaloid heliotrine (PA), and adults captured in the forest (WILD). Groups are compared to controls elsewhere.

	LARVAE	REARED[t]	SUGAR	PA	WILD
Eaten (%)	91	98	96	15	24
Ignored/Nudged (%)	6	2	4	43	31
Distress Behaviors (%)	16	1	1	46	36
Mean Handling Time (sec.)	11.9	8.7	5.2	14.5	14.4
Mean Response Score	2.8	3.0	2.8	1.6	1.8

Note: [t] Category represents combined data from two experiments using P. fulvescens adults reared on S. brenesii offered only water ("P. f." from Table 3-3 and "LAB" from Table 3-7).

Table 3-10. Comparison of ithomiine P. fulvescens treatments offered the Blue Crowned Motmot (Momotus momota). Treatments are: larvae, adults reared on S. brenesii (REARED), similar adults fed sucrose (SUGAR) or sucrose plus the pyrrolizidine alkaloid heliotrine (PA), and adults captured in the forest (WILD). Groups are compared to controls elsewhere.

	LARVAE	REARED[t]	SUGAR	PA	WILD
Eaten (%)	75	96	98	34	25
Ignored/Pecked (%)	14	4	2	24	28
Distress Behaviors (%)	13	2	3	31	28
Mean Handling Time (sec.)	13.2	4.5	4.8	14.4	14.1
Mean Response Score	2.5	3.0	3.0	2.1	2.0

Note: [t] Category represents combined data from two experiments using P. fulvescens adults reared on S. brenesii offered only water ("P. f." from Table 3-4 and "LAB" from Table 3-8).

response scores. These two groups are nearly indistinguishable. Heliotrine butterflies were rejected in 9% more cases, were ignored/pecked in 4% fewer encounters, and elicited 3% more distress behaviors.

Sceloporus malachiticus vs. Momotus momota

Both predator species found P. fulvescens adults reared and offered water or sugar water palatable. They differed, though, in their treatment of P. fulvescens larvae. Both predator species disliked larvae, but to different degrees: while lizards ate 91%, birds ate just 75%. Again, both predator species found heliotrine-fed adults and those captured in forest to be largely unpalatable. They are dissimilar, however, in that lizards preferred forest butterflies to heliotrine-fed ones while motmots did not.

Discussion

Unpalatability of the ithomiine P. fulvescens to vertebrate predators, as hypothesized by Bates and Müller, is conditional for at least the Spiny Lizard (S. malachiticus) and the Blue-crowned Motmot (M. momota). Larvae are unpalatable but rather poorly protected, while adult palatability is variable, possibly contingent upon access to pyrrolizidine alkaloids which confer unpalatability to otherwise palatable adults.

The pyrrolizidine alkaloid-derived adult unpalatability found here is similar to patterns of unpalatability for the

only other predator examined in detail, the large orb-weaving spider Nephila clavipes (Brown, 1984, 1985, 1987; Brown et al., 1991). In this case, recently-emerged ithomiines were palatable for all species tested that use solanaceous larval foodplants (n = 26 species; Brown, 1985). The remaining ithomiine species feed on Apocynaceae (containing pyrrolizidine alkaloids) and are unpalatable to N. clavipes as recently-emerged adults (n = 2 spp.; Brown, 1985). Solanaceous plant feeders, however, became unpalatable to N. clavipes if given pyrrolizidine alkaloids, and to the same degree as most forest butterflies. However, unlike the results of this study, ithomiine larvae feeding on Solanaceae were palatable to N. clavipes (Brown, 1984, 1985).

While unpalatable compared to mealworm controls, individual P. fulvescens larvae stand a poor chance of survival. It is possible that Pteronymia spp. larvae contain enough secondary compounds in their gut to confer unpalatability. Alternatively, these larvae may sequester solanaceous secondary compounds into their tissues. The source of the unpalatability may be a *de novo* synthesis of deterrents or incorporation of compounds from S. brenesii, their larval foodplant. As a family, solanaceous plants contain a host of alkaloids, as well as bitter steroids, terpenes, saponins, and phenolic glycosides (Brown et al., 1991). The ithomiine genus Pteronymia specializes on an especially high-alkaloid content Germinata section of the

genus Solanum (Bradley et al., 1979; Brown, 1987). In any event, deterrent compounds do not appear to make it through pupation intact, as recently-emerged adults are clearly palatable. Other ithomiine species may effectively store predator deterrents from larval foodplants, perhaps even passing them to adults (Brown, 1987), but my feeding experiments with P. fulvescens provide the first evidence of larval protection in any ithomiine species.

Despite the obvious low survivorship, the protective function of deterrents in immature ithomiines may be further enhanced by the natural history of P. fulvescens larvae. Eggs are laid in clusters ranging from 11 to over 150 (pers. obs., Haber, 1978), and larvae feed gregariously until the final instar. Even then, larvae are commonly found associated rather closely on adjacent leaves or branches (pers. obs.). As previously mentioned, larvae are brown, white, and yellow. Although probably somewhat cryptic when feeding on the undersides of leaves, P. fulvescens larvae may be recognizable to a vertebrate insectivore discovering a group or individual larvae in close proximity. I suspect some of the lizards and birds were capable of recognizing P. fulvescens larvae and eventually rejected them on sight (scored as ignored). The advantage of unpalatability in P. fulvescens larvae, therefore, may not lie in surviving an attack but in protecting kin nearby, as originally suggested by Fisher (1928). The larvae of the majority of ithomiines

species, however, are cryptic and solitary. The degree of protection offered to these larvae is poor at best, despite some unpalatability. Pyrrolizidine alkaloids, such as heliotrine that protected adults in this experiment, occur in ithomiine larvae that feed on Apocynaceae. These species tend to be both aposematic and gregarious as larvae (DeVries, 1987)

Adult P. fulvescens emerge palatable to the vertebrate predators studied here. This is wholly different from the chemical defense system of other butterflies, in which recently emerged adults are either protected by secondary compounds sequestered as larvae, as with danaines and some nymphalines, or by *de novo* synthesis, as in heliconiines (all Nymphalidae; Brower, 1984). Ithomiines, like P. fulvescens, must acquire or make defensive compounds later. I found no evidence for defensive compounds produced in the days adults were maintained on either water or sucrose. It is possible that substances acquired by the adult from sources in the environment were absent, however, and *de novo* synthesis could not proceed. To date, the only secondary compounds found in ithomiine adults are varying amounts of a few pyrrolizidine alkaloids. Here, the pyrrolizidine alkaloid heliotrine imparted unpalatability to recently emerged adults. Forest butterflies used in these feeding trials were not tested for presence of pyrrolizidine alkaloids, but other P. fulvescens from the same dates and

localities show varying amounts of several pyrrolizidine alkaloids (see Chapter 4).

Adult acquisition of unpalatability and its reliance on sources of pyrrolizidine alkaloids has fundamental ecological and evolutionary consequences (Brown, 1987; Brown et al., 1991). Ecological consequences are discussed in Chapter 4. Evolutionarily, adult acquisition may be risky while offering a chance of renewal. Perhaps most importantly, adults may be subject to greatest predation at the point in their lives when the impact of natural selection is strongest, that is just prior to reproduction. In all likelihood, ithomiines originally utilized apocynaceous larval hostplants (Edgar, et al., 1974; Edgar, 1982; 1984; Trigo and Motta, 1990) from which they, presumably, acquired pyrrolizidine alkaloids that protected them from predators, as do ithomiines from primitive genera today (Brown, 1987; Brown et al., 1991).

Several hypotheses for a shift in hostplant use to solanaceous foodplants (with concomitant loss of protection immediately post larval stage) deal exclusively with the benefits of such a switch, among them competition by herbivores (Edgar et al., 1974), and increasing chemical diversification within the Apocynaceae that required costly detoxification. The obvious potential cost to adults is the risk associated with unprotected adults at emergence and their use of potentially limited pyrrolizidine alkaloid sources. This cost may be small if pyrrolizidine alkaloids

sources are abundant and predictable (see Chapter 4). Adults may acquire pyrrolizidine from both living and dead plants, and even from other insects (Masters et al., 1983; Boppre, 1986). In addition, females may gain pyrrolizidine alkaloids from spermatophores even in the absence of immediate plant supplies. However, even if an unpredictable defense, recently emerged adults may be protected by mimicry for a time until pyrrolizidine alkaloids become available to them.

A potential benefit to the adult acquisition of defensive compounds found here is emancipation from variable larval foodplant secondary chemistry. An additional benefit is the chance for renewal of lost defense compounds. Butterflies that rely on larval-acquired defenses in adult stages may have fixed or declining concentrations of defensive compounds (Alonso-Mejia, 1991). In such cases, the level of protection is determined in part by maternal oviposition-site selection, larval mobility, and larval foodplant choice. Monarch butterflies (Danaus plexippus; Nymphalidae: Danainae) that acquire cardenolides from asclepiads (Brower, 1969) may concentrate cardenolides, but have little control over the total cardenolide amount sequestered when placed on extremely low cardenolide plants and no control when on asclepiads lacking cardenolides (Brower, et al., 1967; Brower and Fink, 1985). As many asclepiad species are geographically restricted (Woodson, 1947), and asclepiad species exhibit different types and

amounts of cardenolides (Seiber et al., 1986) local populations may range from largely protected to almost entirely palatable (Brower et al., 1968). This is also the case for the closely-related Queen, D. gilippus (Ritland, 1991). In addition to uncertainty in larval foodplant chemistry, monarchs face declining cardenolide content with age (Alonso-Mejia, 1991). In such precarious circumstances, one might expect monarchs to have a defense that allows adults to compensate for low larval protection or cardenolide loss. In fact, they do. Monarchs and other danaines acquire pyrrolizidine alkaloids as adults in the same manner as ithomiines (Brown, 1985; Kelley et al., 1987). In this way, low cardenolide monarchs may have a second chance at unpalatability. If solanaceous foodplants are similarly variable, adult-acquired pyrrolizidine alkaloids may compensate for solanaceous foodplants that are unreliable. In addition, pyrrolizidine alkaloids are used in courtship by many ithomiine and danaine species. Chemicals derived from pyrrolizidine alkaloids are disseminated by males. Females also place pyrrolizidine alkaloids in eggs (Brown, 1984, 1985). In either sex, as pyrrolizidine alkaloids are lost, adult acquisition allows for constant renewal.

Differences in the responses of S. malachiticus and M. momota should not be dismissed. P. fulvescens larvae were more susceptible to the lizards tested than to birds. Conversely, lizards tested here found P. fulvescens adults

that consumed similar amounts of heliotrine to be more unpalatable than their avian counterparts. This last finding may reflect a lower taste threshold for heliotrine among the lizards than motmots (see Chapter 2). In any event, responses of predators to P. fulvescens were variable despite an overall agreement in basic trends. Such trends may differ for other vertebrates predators, as they do for the spider N. clavipes (Brown, 1984) and fawn-breasted tanagers (Pipraeidea melanonota) that readily feed on large numbers of ithomiines (Brown and Vasconcellos-Neto, 1976). Different ithomiine species are also likely to influence predator responses, particularly for those whose larvae specialize on widely different hostplants (Brown, 1987). Finally, non-ithomiine alternative prey are likely to impact a predator's foraging decisions.

It seems Bates and Müller were largely correct in assuming ithomiine unpalatability. Still, palatability is not an inherent property of ithomiines. Instead, there is variation in palatability that is determined, in part, by life stage and exposure to plant secondary compounds as adults. Also important in ithomiine chemical defense is the attacker: both predator species and individual predators within a species show variation in their handling of identically protected butterflies. Variable palatability is sure to influence the degree to which ithomiines act as models in mimicry systems. Therefore, the ecological outcome

of ithomiine unpalatability and its influence on the evolution of butterfly convergence in color patterns, while still a premier example of natural selection, is more complicated than originally proposed over a century ago.

Summary

The chemical protection of an ithomiine species against sympatric vertebrate insectivores, as explicitly stated by Bates and Müller in their theories of mimicry, is shown experimentally for the first time here. In addition, I confirm the ability of pyrrolizidine alkaloids, found in ithomiines, as agents of protection. The ithomiine, P. fulvescens, was offered to Spiny Lizards, Sceloporus malachiticus, and Blue-crowned Motmots, Momotus momota. P. fulvescens were reared on Solanum brenesii (Solanaceae) and offered to predators as larvae, and as adults fed water, a sucrose solution, or a sucrose solution with heliotrine (a pyrrolizidine alkaloid). In addition, wild-caught P. fulvescens were presented. On average, S. malachiticus and M. momota found larvae less palatable than mealworm controls. M. momota was more likely to reject larvae than S. malachiticus. Both predator species ate reared adults fed water or sucrose as readily as the palatable Anartia fatima. Heliotrine made P. fulvescens as unpalatable as forest P. fulvescens for M. momota, and slightly more unpalatable than forest individuals with S. malachiticus. For P. fulvescens,

some protection was available to larvae, but freshly-emerged adults were palatable. Adults gained unpalatability after acquiring heliotrine, a pyrrolizidine alkaloid similar to those found in most ithomiine species.

CHAPTER 4
SOURCE AND OUTCOME OF VARIABLE DEFENSIVE CHEMISTRY IN A MÜLLERIAN ASSEMBLAGE

Introduction

Sources and outcome of variation in species interactions are rarely explored (Thompson, 1988). It is this variation, however, upon which natural selection acts to impart an evolutionary change. One system in which the sources and outcome of variation have been studied intensively is chemical defense in the monarch butterfly (Danaus plexippus, Nymphalidae: Danainae; see Brower, 1984). Monarch individuals differ in cardenolide type and amount (Brower 1969). The source of variation is both larval foodplant chemistry (Seiber et al., 1986) and butterfly age (Alonso-Mejia Mejia, 1991). The outcome of the variation is differential chemical defense (Brower, 1969), referred to as automimicry (Brower et al., 1968). Variable defense among other species that mimic the monarch may change both the speed and direction of color pattern selection for the monarch (Ritland and Brower, 1991). Still, the sources and outcome of variable defense have not been explicitly measured for mimetic assemblages with many species.

Sources and Outcome of Variable Defensive Chemistry in Ithomiine Butterflies

Here, I extend the study of variation in chemical defense to the Ithomiinae (Nymphalidae). These neotropical butterflies show considerable quantitative variation in chemicals used for defense (Brown, 1985; Chapters 2 and 3) and belong to mimicry complexes with large numbers of species (Bates, 1862). I examine ithomiine ecological chemistry (Brower, 1969) to uncover patterns of variable defense. Further, I use a mathematical model of mimicry (Brower et al., 1970) to measure how variable defense and mimicry determine predation risk. In doing so, I assess both the effectiveness of variable defense as an antipredator strategy and its impact on the evolution of mimicry.

Source of Ithomiine Defense

Ithomiine butterflies contain pyrrolizidine alkaloids that, in sufficient quantity, protect ithomiines from arachnid and vertebrate predators (Brown, 1984; 1985; Chapters 2 and 3). A few primitive ithomiine species sequester pyrrolizidine alkaloids as larvae from their apocynaceous foodplants (Edgar 1984; Brown et al., 1991). The majority of species (97%), though, feed on solanaceous foodplants as larvae (Drummond, 1976; Haber, 1978; Drummond and Brown, 1986). Although their larval foodplants are rich in secondary compounds, adults of these species are unprotected until they acquire pyrrolizidine alkaloids from exogenous sources (Brown, 1984, 1985; see Chapter 2).

The ecological chemistry of 97% of ithomiine species is adult sequestration of pyrrolizidine alkaloids and the dual role of these defense compounds as mate attractants (Brown, 1984). Males of most ithomiine species obtain pyrrolizidine alkaloids from plant nectars and rotting plant parts (Boppre, 1986). The majority of females, on the other hand, probably acquire most of their pyrrolizidine alkaloids from spermatophores obtained in mating (Brown, 1985). In addition to defense, male ithomiines also use pyrrolizidine alkaloids as precursors for pheromones that act as mate attractants (Edgar, et al., 1976). Females may protect their eggs with pyrrolizidine alkaloids, as well (Brown, 1985).

Variation in Ithomiine Defensive Chemistry

Brown (1984, 1985, 1987) showed that five pyrrolizidine alkaloids varied in amount for 134 species from 322 non-overlapping populations. Likely patterns of pyrrolizidine alkaloid variation include differences between species, sex, age (Brown, 1985) and season (Trigo and Brown, 1990). Species level patterns may be phylogenetic, reflecting generic or higher taxonomic patterns of larval foodplant, larval aposematism, and adult size (Brown, 1985). Additionally, species may differ if pyrrolizidine alkaloid use or loss is mediated by species-level processes, such as intensity of sexual selection, egg size and number, differential predation, parasitism, and so on. Sexes may have different principal pyrrolizidine alkaloid sources, and

may also differ in their rate of pyrrolizidine alkaloid loss: males volatilize the alkaloids for pheromones, females place them in eggs (Brown, 1985). Age is a potential source of variation for two reasons. First, newly emerged adults of most species have no pyrrolizidine alkaloids and accumulation may take time. Second, loss may increase with age as a result of metabolism or scale loss, both of importance for the monarch and its protective cardiac glycosides (Alonso-Mejia, 1991). Seasonal accessibility to pyrrolizidine alkaloids is likely, as well, if ithomiines depend largely upon access to dried plant parts or floral nectars that are phenologically restricted.

Interactions

The effects of species, sex, age, and season are not independent categories. For instance, species may differ from one another if they exhibit different population age structure. Alternatively, sexes may react differently to seasonal variation in plant sources. The great potential for interactions, therefore, requires that all factors be tested separately and simultaneously.

Current Evidence

Unfortunately, former studies of ithomiine chemical defense do not consider species, sex, age, and season in this way (Brown, 1984; Brown, 1985; Trigo and Brown, 1990). Instead, they combine samples from different geographical locations and seasons. Comparisons restricted to a single

species, location, or time are often too small for meaningful comparison (Trigo and Brown, 1990). Perhaps as a result, trends emerging from ithomiine species, age, and sex are sporadic and remain inadequately tested.

Predicting the Outcome of Variable Defense

The impact of variable defense is complicated by mimicry. An experience with an unpalatable individual may cause a predator to skip some number of ithomiines before resampling (Brower, 1984). The importance of variable chemical defense and predator learning to the operation of chemical defense has been modelled (Brower et al., 1970; Pough et al., 1973; Owen and Owen, 1984). I use the model of Brower et al. (1970) which assimilates the proportion of unpalatable prey, memory span of predators, and abundance of prey relative to predator and estimates the increased likelihood of escaping predation as the result of variable defense and mimicry. This model was developed for a single species. For the first time, I extend it to allow comparisons of predation risk between species, sexes, ages, and seasons.

The Model

In short, the model allows me to calculate the probability of survival if all ithomiines are palatable, expressed as:

$$j_o = 1 - n/m,$$

where n = number of ithomiines eaten per time and m = the number of ithomiines available to the predator, in this case a single Blue-crowned Motmot (Momota momotus).

I next compare the case where all ithomiines are palatabile to that when some proportion of ithomiines are unpalatable, k'. In this situation, each bird eats at least one ithomiine. If a bird eats an unpalatable ithomiine it stops foraging on them. Some birds, however, encounter a palatable ithomiine and go on to eat a second. This is equivalent to $(1 - k')$. A subset of birds encounter another palatable ithomiine $(1 - k')^2$, and so on, so that the average number of ithomiines eaten by a bird is a geometric series = $[1-(1 - k')^n / k']$. Here, the number of ithomiines eaten is a function of the proportion of unpalatable ithomiines, k', and ithomiine density, m. The likelihood of survival for an ithomiine in this circumstance is written as:

$$j_{k'} = 1 - \{[1 - (1 - k')^n]/mk'\}.$$

The difference between $j_{k'}$ and j_o is the increased likelihood of survival resulting from variable chemical defense and mimicry. This is the mimetic advantage , or ΔJ.

Assessing the Importance of Variable Defense in Ithomiines

I substitute values of m (number of ithomiines) and k' (proportion of unpalatable ithomiines) from data obtained in this study for a range of likely n (the number of ithomiines consumed by a predator in the absence of chemical defense). Using the model, the predation risk for members of different

ithomiine groups, including species, sexes, or ages, may be compared to see which suffers or gains most when there is variable defense with mimicry. I test those groups that show a difference in relative abundance and pyrrolizidine alkaloid content. For instance, if sexes are found to differ in proportion of palatable individuals, I use abundance data and the model to measure the impact of this difference in terms of predation risk. I could even extend the model to see in which species or at what season the sex difference had the greatest effect. Finally, I appraise the relative direction and strength of color pattern evolution based upon the relative risk of predation and tenets of classical mimicry theory (Huheey, 1988).

The Current Study

Here, I report the 1987 annual pattern of changing abundance and chemical variation for all tawny ithomiines within a Costa Rican ithomiine assemblage. I seek to explore likely sources of pyrrolizidine alkaloid variation, including plant flowering phenology. I expose patterns of variation in ithomiine defensive chemistry, as well. I test differences between ithomiine species, sexes, ages, and with season. Variable pyrrolizidine alkaloid content, in turn, is transformed into variable palatability using taste rejection thresholds measured with the Blue-crowned Motmot (Momotus momota). Finally, I use the model of automimicry developed

by Brower et al. (1970) to incorporate ithomiine abundance and variable palatability, illustrating: (1) how ithomiine ecology and mimicry ameliorate potential inadequacies inherent in variable defense, and (2) the uncertain direction of color pattern evolution as a result of inconsistent protection.

Materials and Methods

Description of Study Site

Location and Climate

Butterflies were collected along the Rio Guacimal in Monteverde de Puntarenas, Costa Rica. Monteverde is transitional wet-moist forest in the Cordilla de Tilaran at 1400 m. Unlike more typical tropical wet forests (e.g., Drummond, 1976), Monteverde has a distinct wet and dry season (see Figure 4-1 for a typical profile of monthly rainfall. This is for 1987-1988, the year I collected ithomiines for this chapter). In Monteverde, the wet season usually begins in the middle of May and extends into November. From May through August, heavy rainfall is normally restricted to the late afternoon. From September to January, rains come as mist with wind for most of the day, but there is usually a short amount of sun in early to mid-morning (pers. obs.). Precipitation decreases from October to mid May. By the end of January there are dry, sunny conditions with few days of

rain, and this pattern extends into February until the last half of May.

Study Site

Distribution of ithomiines along the Rio Guacimal in Monteverde is extremely localized but consistent from year to year. Haber (1978) collected ithomiines in the same characteristic "ithomiine pocket" (sensu Brown and d'Almeida, 1970) in which I collected ithomiines in 1983, 1984, 1986, and in this study. All collecting occurred in a single location between two sharp turns in the river.

Mimetic Relationships

Haber (1978) delineates five mimetic complexes for this and other ithomiine assemblages: (1) clearwing (wings transparent with black margins, black veins, and white subapical forewing stripe), (2) black and yellow (wings black with bright yellow bars), (3) tiger (wings orange with transverse black bars and yellow subapical forewing stripes), (4) golden-translucent (wings translucent golden brown with smokey black shading), and (5) black and rust (forewings black with small yellow or white spots, hindwings rust colored with black margins). All occur in Monteverde, but black and yellow ithomiines are extremely rare (see below). Of the remaining, I argue there is one abrupt difference in color pattern that occurs between clearwings and the other three color pattern types. Tiger, black and rust, and

golden-translucent ithomiines are not distinct groups in Monteverde. Many species represent intermediate cases. Therefore, here I combine all ithomiines of species that grade from gold to rust, with black, white, and yellow stripes, spots, or bars into a single mimetic complex I refer to as "tawny."

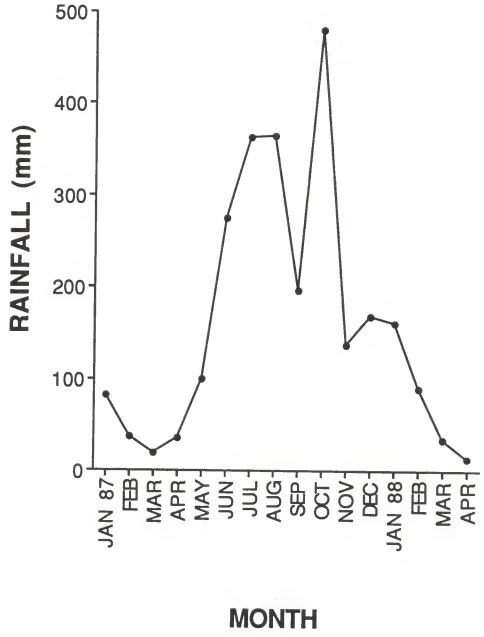
Butterfly Collections

I examined the Rio Guacimal site from February 1987 to May 1988. When present, butterflies were netted monthly. At least five consecutive sunny days in the last two weeks of each month were collecting days. On these days, up to twenty tawny butterflies were randomly collected. Hence, I collected 100 tawny ithomiines per month (when available). Subsequent days I collected non-randomly to boost numbers of rarer species or sexes. Butterflies were never collected in the first two weeks of a month, separating monthly samples by at least 14 days.

Patterns of Ithomiine Abundance.

Absolute and relative measures of ithomiine abundance were made so that I could calculate population sizes for ithomiine species, and numbers of each sex, age, and with season. First, I estimate the exact number of ithomiine butterflies each month by mark-release-recapture techniques. After the five collecting days, I selected the most abundant

Figure 4-1. Annual pattern of monthly rainfall for Monteverde, Costa Rica in 1987 and early 1988. Rainfall data are courtesy of John Campbell.


RAINFALL (mm)
500
400
300
200
100
0
JAN 87
FEB
MAR
APR
MAY
JUN
JUL
AUG
SEP
OCT
NOV
DEC
JAN 88
FEB
MAR
APR
MONTH

species and marked butterflies of this species along the margin of the forewing with yellow correcting fluid. Spots were not noticeable to me before recapture. I used Bailey's triple catch method (Southwood, 1978) that requires two unique marks on subsequent days. Thus, marks were first placed on the right dorsal forewing and on the following day on the left forewing.

I then measured the relative abundance of different species, sexes, and ages by scoring these factors for the first 100 individuals captured each month (where possible). This allowed me to extrapolate absolute abundance measures from the most common species each month to other species, sexes, and ages that month. The underlying assumption, of course, is that the likelihood of capture was not a function of ithomiine species, sex, or age class. Species were identified with dichotomous keys and species descriptions (Fox, 1940), museum specimens (Allyn Museum, Sarasota, FL), and a fieldguide (DeVries, 1987). Sexes were determined by the presence of androconial hairs in males. Ages were determined subjectively by the relative amount of wing wear.

Chemical Analyses

Isolation of Pyrrolizidine Alkaloids.

Upon capture, butterflies were placed in glassine envelopes. After the daily collection, individuals were sexed and identified to species. Next, butterflies were

returned to envelopes and were frozen at 0° C. Butterflies were kept frozen until chemical analyses or feeding experiments.

All ithomiines were examined for pyrrolizidine alkaloid content using a method modified from Mattocks (1986). Butterflies were examined individually for total pyrrolizidine alkaloid content. Each was dried at 60 ° C for 12 hours and weighed. Next, single butterflies were macerated in 4 ml methanol. The resultant homogenate was filtered through glass wool. Solids were discarded, containing largely chitinous portions of the exoskeleton. Filtrate was evaporated under nitrogen and resuspended in 4 ml 2N H_2SO_4. To remove fats, the resuspended fraction was extracted three times with equal volumes of chloroform. To the H_2SO_4 fraction I added 400 mg powdered zinc and kept the mixture shaking on ice at least 3 hours. This reduces all pyrrolizidine alkaloids in N-oxide form to their free base analog. Zinc was removed by filtration through glass wool and the resultant acidic solution was basified with NH_4OH to pH 10. The solution was next washed with four equal volumes of chloroform. At this pH, alkaloids are neutral and soluble in chloroform. To facilitate this, the basic solution was first saturated with NaCl. Chloroform was evaporated under nitrogen. Isolated pyrrolizidine alkaloids were frozen until analysis.

Quantifying Pyrrolizidine Alkaloid Content

Individual butterflies were assayed for total pyrrolizidine alkaloid content using thin layer chromatography and densitometry. Pyrrolizidine alkaloids isolated from individual butterflies were resuspended in 50 µl methanol. Merck 20 x 20 cm, 0.25mm thick, F_{254} µm 60 Å silica gel TLC plates were activated by heating at 100° C for 30 minutes, plates were cooled in a desiccator. Samples (5 µl) were then spotted 2 cm apart onto the plate. Because I was interested in only the total amount of pyrrolizidine alkaloid per butterfly, individual pyrrolizidine alkaloids were not separated in a solvent system. In total, each plate contained 48 spots that corresponded to ithomiine samples, 8 additional spots of 10 µg monocrotaline and 8 spots of 50 µg monocrotaline (free base form, Aldrich Chemical, 99% pure, lot A7130).

Plates were allowed to air dry before development. Plates were then sprayed with 1% o-chloranil in toluene and heated at 100° C for 5 minutes (Molyneux and Roitman, 1980). After cooling, plates were sprayed with Ehrlich's reagent (2.0 g p-dimethylaminobenzaldehyde in 100 ml absolute ethanol containing 2.0 ml boron trifluoride etherate). Plates were again heated at 100° C for 20 minutes. Pyrrolizidine alkaloids then visualize as bluish-brown spots on an orange background. Plates were placed under continuous fluorescent

lighting for 48 hours. The outcome was purple-brown spots on a pale yellow background.

Spots were quantified with a KONTES Model 800 Scanning Densitometer operated at a single wavelength (560 nm). A standard curve was based upon 10 samples of 10, 50, 100, 250, 500, and 1000 µg free base monocrotaline, taken through the extraction steps listed above. The presence of pyrrolizidine alkaloids was also detected with GC-mass spectroscopy. Butterfly samples were placed in 25 µl HPLC grade chloroform of which 1 µl was injected. I used a Perkin Elmer Ion Trap Detector, Perkin Elmer Capillary GC 8420 with a splitless injector at 270° C, programmed to increase 10° C per minute from 50 to 300° C.

Statistical Consideratons

Sample sizes varied with ithomiine availability (see below). I limited statistical comparisons to those groups with greater than 10 observations per category. Distributions departed significantly from normal in most cases (Fisher's Normality Test, $p < 0.05$) so non-parametric tests were used exclusively. Sample size and variance sometimes varied greatly between species, weakening the power of tests like the Kruskal Wallace ANOVA (Day and Quinn, 1989). When using the Kruskal Wallace ANOVA, I often followed with the Dunn's Nonparametric Multiple Comparison Test that incorporates unequal sample size and variance (Zar,

1984). The Kruskal Wallace ANOVA tests medians, the Dunn's tests mean ranks. These tests are independent and, partly because of differential sensitivity to sample size and variance, sometimes give different results.

Feeding Experiments

Three Blue-crowned Motmots (Momota momotus) of undetermined age and sex were used to determine a palatability threshold based on pyrrolizidine alkaloid content in wildcaught ithomiines. Three birds were captured in mistnets and housed in 1.2 x 2.54 cm galvanized wire cages measuring 61 x 61 x 50.8 cm. Birds were maintained on a mixture of chick feed, carrot, hard-boiled egg and oil for one week. Birds were then offered wildcaught Pteronymia fulvescens (Ithomiinae) captured in May 1988.

Butterflies were captured the day before presentation to birds and frozen. Butterflies were thawed and placed with wings appressed laterally in a petri dish. Birds were left with the dish of butterflies for two hours, after which the laboratory diet was resumed. Butterflies that were not eaten were collected and frozen for later analyses. This was repeated for six days, during which each bird was offered 60 P. fulvescens. Each day, a group of 10 P. fulvescens were similarly thawed, left for two hours outside of the cages and then kept in the freezer for later chemical analysis. At the end, I had three samples of P. fulvescens, each representing

those rejected out of 60 butterflies for a given bird. I also had a fourth group of 60 P. fulvescens that acted as a control.

Floral Census

Flowers known or presumed to contain pyrrolizidine alkaloids were censused monthly throughout the area when ithomiines were present. Areas surveyed were 3 kilometers of roadside and four 5 x 5 m portions of pasture. These represented the nearest pyrrolizidine alkaloid sources to the ithomiine assemblage that I could locate. Ithomiines were only occassionally seen on these plants so that ithomiine censuses on plants were not possible. Plant families reported to contain pyrrolizidine alkaloids include the Apocynaceae, Asteraceae, Boraginaceae, Leguminosae, Orchidaceae, Ranunculaceae, and Scrophulariacea (references in Mattocks, 1986). I estimated flower number by counting flowers or counting areas of flower heads and estimating flower number for the entire inflorescence.

Results

Assemblage Size and Species Composition

Ithomiine species predominated in the collecting area, although several other butterfly species were present; most abundant were Morpho peleides limpida (Nymphalidae:

Morphinae), Heliconius erato (Nyphalidae: Heliconinae), and Epiphile adrasta (Nymphalidae: Nymphalinae), all distinctly different in color and flight behavior than the ithomiines studied here. Non-ithomiine Batesian mimics are reported for the same collecting site (Haber, 1978; DeVries, 1983) but I never observed them.

Seasonal Changes in Ithomiine Abundance

From February 1987 to February 1988, the number of ithomiine butterflies in the area fluctuated from at or near zero to over 2000 individuals (Figure 4-2), based on monthly recapture data of the most common species (Table 4-1) and relative abundance data (Table 4-2). Ithomiines appeared in Monteverde in March and increased in number through September, after an initial decrease between May and June. Numbers quickly increased in July and peaked in September. Beyond September, ithomiine numbers decreased quickly until ithomiines were absent in January and February.

Common vs. Rare Species

Species were either common in collections ($n > 100$ total individuals per species collected in random samples for all months combined) or occurred in very small numbers ($n \leq 11$ total in samples; Table 4-2). A single rare species never accounted for more than 6% of the ithomiines captured per month, although rare species combined represented up to 23% of the ithomiines collected in a single month (June; Figure 4-3). Common species, on the other hand, varied in relative

Figure 4-2. Number of tawny ithomiines of 20 species along the Rio Guacimal, Monteverde de Puntarenas, Costa Rica from January 1987 to March 1988. Monthly number is based upon mark-release-recapture data for the most common tawny species captured each month. Relative abundance of all tawny species was obtained by randomly collecting 100 individuals each month. Total number of ithomiines per month was obtained by extrapolating the population size of the most common species with values of relative abundance .

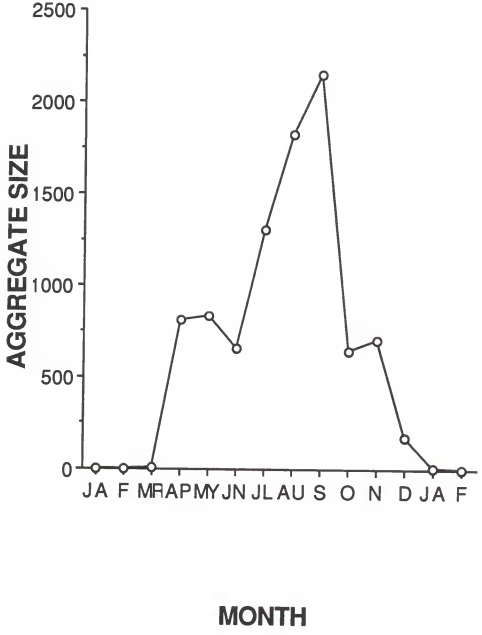

2500
2000
1500
1000
500
0
AGGREGATE SIZE
JA F MRAPMYJN JLAU S O N D JA F
MONTH

Table 4-1. Population size of the single most abundant species in each month. Butterflies were netted, marked with yellow paint on the forewing, and released. Marking occurred for three days with the number captured on each or previous days recorded. Population size was determined by Mark-release Recapture and calculation with Bailey's Triple Catch Method for small sample sizes (Southwood, 1978).

Month	Sp.	Marked on Day 1	Marked on Day 2	Marked on Day 3	Recaptured on Day 2	Recaptured on Day 3: 1	Recaptured on Day 3: 2	Recaptured on Day 3: 1+2	Pop. Size
April	P.f.	164	139	61	30	19	18	4	470
May	P.f.	155	122	47	27	16	15	5	495
June	I.h.	79	42	37	27	27	8	2	278
July	I.h.	82	66	47	15	7	6	3	411
August	I.x.	117	109	92	32	9	6	6	599
Sept.	H.eu.	168	129	78	21	8	7	3	879
Oct.	H.eu.	29	27	25	10	8	4	1	335
Nov.	I.x.	26	30	21	10	4	1	0	224
Dec.	I.x.	24	16	14	3	4	2	0	107

Table 4-2. Composition of Monteverde ithomiine assemblage for 1987. Numbers are from the first 100 randomly sampled individuals captured, except for December when 58 total individuals were collected. Numbers in parentheses are for species captured in subsequent samples but not represented in the initial 100 captured. Species are listed from most to least common. Species richness and species diversity (Shannon-Weiner H') are listed below each month. Numbers in parentheses are not included in totals, species richness, nor species diversity calculations.

	NUMBER IN COLLECTION by MONTH (number of males number of females)									
SPECIES	AP	MY	JN	JL	AU	S	O	N	D	TOTAL
Ithomia heraldica	21	4	33	21	15	20	16	22	9	
	4	2	9	11	10	8	4	5	6	220
Ithomia xenos	9	1	17	15	15	2	1	13	19	
	0	1	1	15	18	11	1	19	18	176
Pteronymia fulves.	44	51	0	(1)	1	0	2	1	0	
	14	34	1	0	6	1	0	1	0	156
Hypothyris euclea	0	0	0	0	1	15	23	5	0	
	0	0	0	0	10	25	31	19	0	129
Pteronymia notilla	0	1	9	16	7	8	10	10	2	
	1	2	7	5	8	5	4	4	4	103
Hyalyris excelsa	(2)	0	4	1	0	0	1	0	0	
	(1)	(2)	3	0	0	0	3	0	0	12
Hyposcada virgini.	(1)	0	2	1	0	1	0	1	0	
	0	(2)	0	3	1	2	0	0	0	11
Dircenna relata	3	0	0	1	1	0	0	0	0	
	0	1	2	2	0	0	1	0	0	11
Napeogenes tolosa	0	0	0	1	1	0	1	0	0	
	2	0	1	1	2	0	0	0	0	9
Mechanitis lysimnia	0	0	1	2	2	0	0	0	0	
	0	2	0	0	1	0	0	0	0	7
Dircenna klugii	(4)	0	6	0	0	0	0	0	0	
	0	(2)	0	0	0	(1)	0	0	0	6
Mechanitis menapis	1	0	0	0	0	(1)	0	0	0	
	0	0	2	2	0	(1)	0	0	0	5

Table 4-2--continued

SPECIES	NUMBER IN COLLECTION by MONTH (number of males number of females) AP	MY	JN	JL	AU	S	O	N	D	TOTAL
Mechanitis polymnia	0	0	(1)	1	1	0	0	0	0	
	1	0	0	0	0	0	0	0	0	3
Melinaea ethra	0	0	1	0	0	0	0	0	0	
	0	(1)	0	2	0	0	0	0	0	3
Eutresis hyperia	0	0	0	0	0	0	2	0	0	
	0	0	0	0	0	0	2	0	0	2
Tithorea tarricina	0	0	0	0	0	0	0	0	0	
	(1)	0	0	0	0	1	0	0	0	1
Dircenna chiriquen.	0	0	1	0	0	0	0	0	0	
	0	0	0	0	0	0	0	0	0	1
Dircenna olyras	0	0	0	0	0	0	0	0	0	
	0	1	0	0	0	0	0	0	0	1
Olyras crathis	0	0	0	0	0	0	0	0	0	
	0	0	0	0	0	1	0	0	0	1
Oleria zelica	0	0	(1)	0	0	0	0	0	0	
	0	0	0	0	0	0	0	0	0	0
TOTAL	100	100	100	100	100	100	100	100	58	
NUMBER OF SPECIES	8	7	13	11	10	8	9	6	3	
SPECIES DIVERSITY	1.1	0.7	1.8	1.7	1.8	1.5	1.4	1.4	0.8	

abundance from month to month but represented at least 20% of the total ithomiines captured in at least one month.

Given small sample sizes for rare species and the relatively minor contribution of any one rare species to the tawny ithomiine assemblage, rare species were ignored in species comparisons. Removing rare species did not influence the seasonal pattern of combined ithomiine abundance noted above. The common species examined in detail, then, were: Hypothyris euclea, Ithomia heraldica, Ithomia xenos, Pteronymia fulvescens, and Pteronymia notilla.

Seasonal Changes in Species Abundance

Species differed widely in their estimated population size (Figure 4-4) and relative abundance (Figure 4-3), both within and between months. Within months, relative abundance showed no consistent pattern. At times, a single species predominated, but most months show two or more principal species. Between months, I. heraldica was most consistently common and P. notilla remained low, but relative ranks varied widely.

For a given species, absolute abundance changed seasonally in ways that generally reflected the assemblage-wide pattern of abundance. The exceptions were Pteronymia fulvescens and Hypothyris euclea. P. fulvescens was most abundant in April and May and was all but absent other months. Conversely, H. euclea was initially absent, appearing first in August, peaking in September, and was

Figure 4-3. Relative abundance of the five most common tawny ithomiine species in Monteverde, Costa Rica. All other species (n = 15) are combined to the category RARE. Common species are those with more than 100 total captures in random samples over the year (Table 2); rare species represented ≤ 11 individuals captured for the same period.

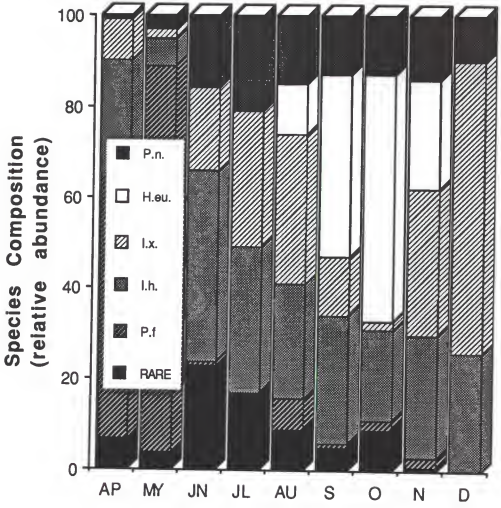
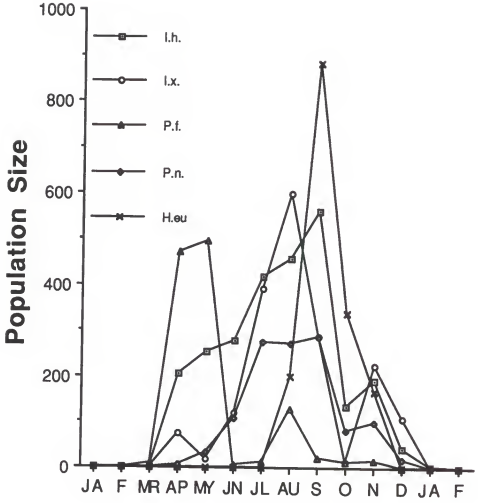

Species Composition (relative abundance)
100
80
60
40
20
0
P.n.
H.eu.
I.x.
I.h.
P.f
RARE
AP
MY
JN
JL
AU
S
O
N
D
MONTH

Figure 4-4. Population sizes of the most common tawny ithomiine species present. Monthly number is a combination of mark-release-recapture and relative abundance data (see text). Ithomiines were absent January to February 1987, 1988.


1000
800
600
400
200
0
Population Size
I.h.
I.x.
P.f.
P.n.
H.eu
JA F MR AP MY JN JL AU S O N D JA F
MONTH

absent again by December. Ithomia heraldica was the most consistently abundant species present in the assemblage. It occurred in all months and exceeded 100 individuals for all months but December, when only 58 total ithomiines were collected.

Relative Abundance of Sexes

For the most part, the sex ratio of ithomiines in the assemblage for all species combined was equal over the season (Table 4-2). The two months where sex ratio significantly departed from unity were April and June (Chi-square test, $p < 0.05$). In these months, there were over three times as many males as females.

Sex ratios among the five common species (Table 4-2) were tested by Chi-square at $p < 0.05$ for months where they were abundant ($n \geq 20$, to give $n \geq 10$ for both sexes expected). Sex ratios were males-biased for I. heraldica in April (21:4), June (33:9), September (20:8), and November (22:5), for I. xenos in June (17:1), and for P. fulvescens in April (44:14). Sex ratio was female-biased in one case, for H. euclea in November (5:16).

Seasonal Age Distribution

Figure 4-5 depicts the relative age distribution, based on wing wear, of the ithomiine assemblage each month. Overall, the age distribution was not uniform (Friedman 2 way ANOVA, $p = 0.001$). Still, the relative abundance of each age class appears relatively stable over time, except that

freshest individuals seemed to increase in September, gradually decreasing toward the end of the year.

Patterns of Variation in Defensive Chemistry

Identity of Defensive Compounds in Adult Ithomiinae

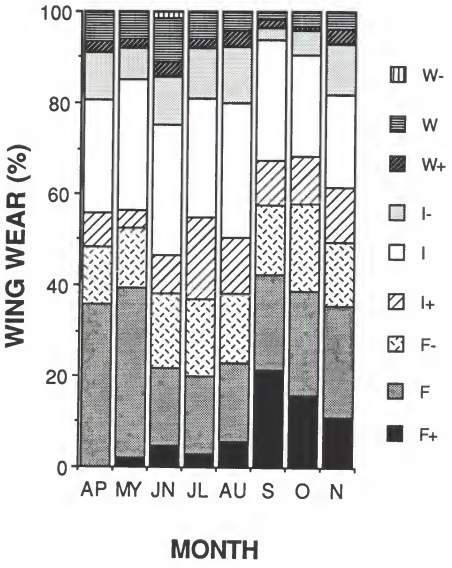
Densitometry after pyrrolizidine alkaloid isolation and TLC could reliably detect 10 µg monocrotaline. GC-mass spectroscopy confirmed the presence of pyrrolizidine alkaloids for 10 ithomiine samples, representing 10 species, all with greater than 100 µg material. Spectra were consistent with the types of pyrrolizidines noted by Brown (1985), namely the dehydropyrrolizidine alkaloids like lycopsamine, intermedine, echinatine, rinderine, and indicine (Figure 2-1).

Quantitative Patterns of Pyrrolizidine Alkaloid Distribution

Total amount of pyrrolizidine alkaloids (µg/individual) and pyrrolizidine alkaloid concentration (% dry wt.) in ithomiines were correlated in a linear fashion ($R^2 = 0.894$, $p < 0.05$; Figure 4-6). Consequently, I report only concentration.

Pyrrolizidine alkaloid variation in ithomiines. The distribution of pyrrolizidine alkaloid concentrations for all ithomiines combined approximated a negative binomial distribution (Figure 4-7). In other words, most ithomiines had little or no pyrrolizidine alkaloids and the frequency of

Figure 4-5. Monthly age distribution, based on wing wear comparisions, for 20 species of tawny ithomiines in a single assemblage in Monteverde, Costa Rica. Ithomiines were classified to wing wear categories upon capture based upon their appearance: F = fresh, I = intermediate, and W = worn. The additional +,- denote further gradations of scale loss.


WING WEAR (%)
100
80
60
40
20
0
AP MY JN JL AU S O N
MONTH
W-
W
W+
I-
I
I+
F-
F
F+

observations decreased in a geometric fashion with increasing concentration.

Between species. Species differed in their median pyrrolizidine alkaloid concentration (KW ANOVA, $p = 0.0001$ for all species; Figure 4-8). Ranked from highest to lowest median concentration for all months combined, the trend for common species was: P. fulvescens, I. xenos, I. heraldica, H. euclea, and P. notilla. Common species, however, were not statistically distinct from one another, based upon mean ranks (Dunn's, $p < 0.05$).

Between sexes. Males and females differed in their concentration of pyrrolizidine alkaloids for all observations combined (MW U test, mean rank males = 575, females = 537, $p = 0.047$). The difference was small, however. Both males and females had modes of 0, medians within 0.01% dry wt., and similar distribution shapes (Figure 4-9). The difference seems to result from relatively few (25 individuals, or 4%) highly concentrated males (above 1.0% dry wt.).

Between ages. Wing wear combined for all species and months was not an indicator of pyrrolizidine alkaloid concentration (Figure 4-10). No significant differences among wing wear categories were found (Kruskal Wallace ANOVA, $p = 0.36$ for concentration) and there were no apparent trends.

Between seasons. Ithomiines differed in their concentration of pyrrolizidine alkaloids depending upon the month they were collected (Kruskal Wallace ANOVA, $p = 0.0001$;

Figure 4-6. Linear relationship between concentration and total amount of pyrrolizidine alkaloids for tawny ithomiine species in Monteverde, Costa Rica. The two measures are related as $y = 0.036 + 0.003x$, $R^2 = 0.894$, ($p < 0.05$).

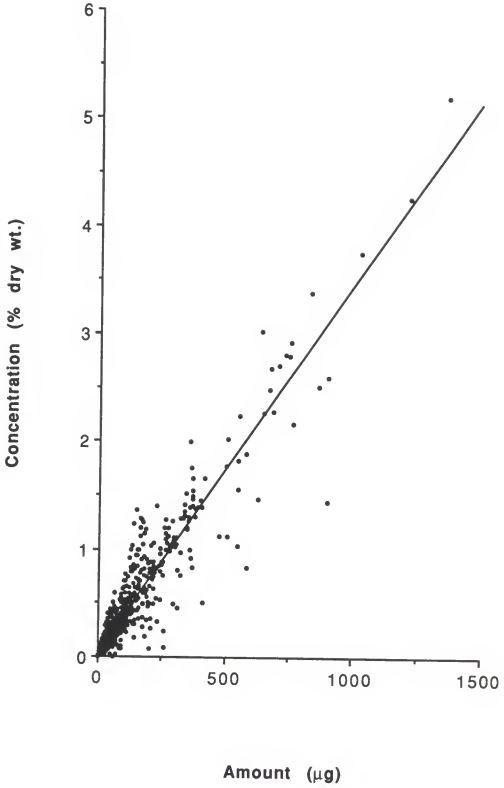

Concentration (% dry wt.)
6
5
4
3
2
1
0
0
500
1000
1500
Amount (µg)

Figure 4-7. Individual variation in pyrrolizidine concentration (% dry wt.) for tawny ithomiine butterflies.(n = 1116) by TLC/densitometry. These values represent 20 species of 13 genera for a total of 1116 individuals. The pattern shows many individuals (> 30%) are completely lacking in pyrrolizidine alkaloids and the frequency in higher categories declines geometrically.

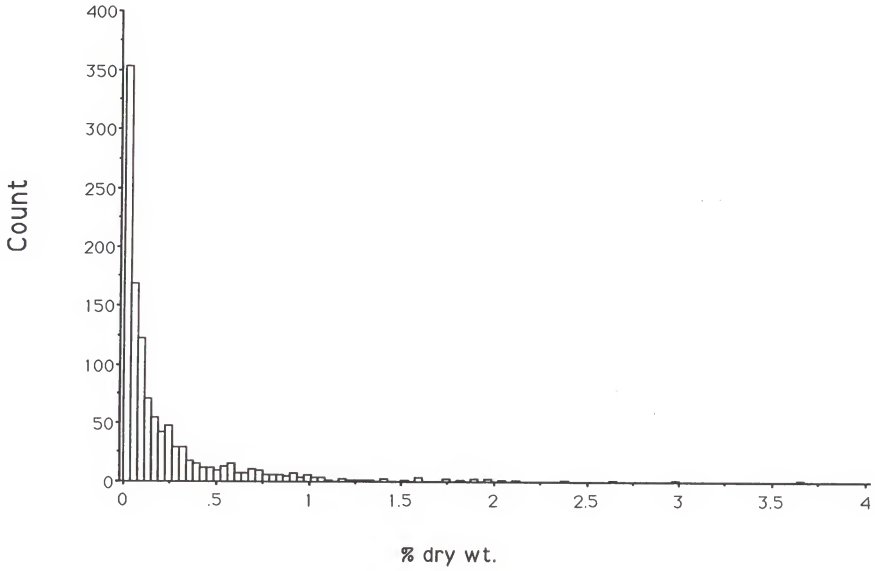

Count
400
350
300
250
200
150
100
50
0
0
.5
1
1.5
2
2.5
3
3.5
4
% dry wt.

Figure 4-8. Species differences in ithomiine pyrrolizidine alkaloid concentration (% dry wt.). Species represent all tawny ithomiines captured from March to December 1987. Species are listed from highest to lowest median. Medians are different by Kruskal Wallace ANOVA ($p = 0.0001$). Descriptive statistics are represented in graphic format by the "box and whiskers" method of Tukey (1977). Here, the median is shown as a horizontal bar, the box incorporates observations between the 25th and 75th percentiles. The lower whisker extends 1.5 times the value from the median to the 25th percentile while the upper whisker is 1.5 times the value from the median to the 75th percentile. Observations beyond this range are plotted as individuals points. Samples sizes are: Eutresis hyperia (Eu) = 2, Olyras crathis (Oc) = 1, Dircenna olyras (Do) = 1, Hyalyris excelsa (Hyx) = 18, Melinaea ethra (Me) = 8, Oleria zelica (Oz) = 1, Pteronymia fulvescens (Pf) = 167, Ithomia xenos (Ix) = 247, Mechanitis polymnia (Mp) = 8, Dircenna chiriquensis (Dc) = 4, Dircenna klugii (Dk) = 14, Mechanitis lysimnia (Ml), Ithomia heraldica (Ih), Hypothyris euclea (Heu) = 133, Mechanitis menapis (Mm) = 10, Dircenna relata (Dr) = 19, Hyposcada virginiana (Hv) = 19, Tithorea tarricina (Tt) = 2, Napeogenes tolosa (Nt) = 9, Pteronymia notilla (Pn) = 123.

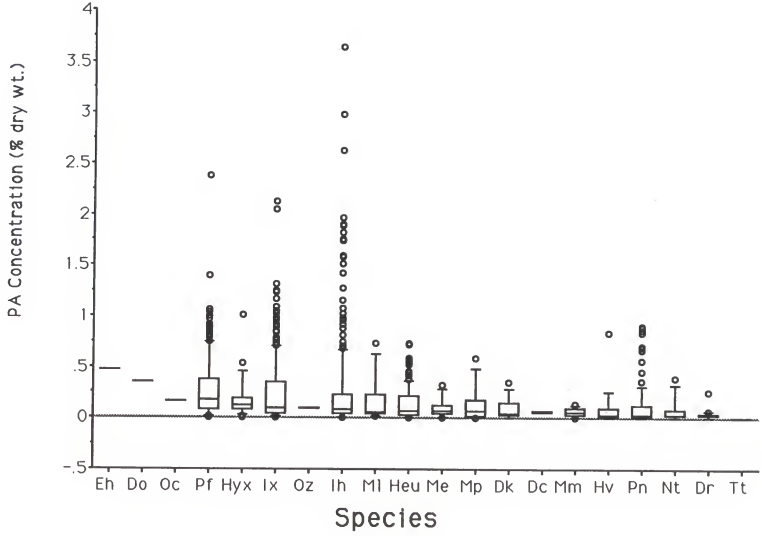

PA Concentration (% dry wt.)
4
3.5
3
2.5
2
1.5
1
.5
0
-.5
Eh Do Oc Pf Hyx Ix Oz Ih Ml Heu Me Mp Dk Dc Mm Hv Pn Nt Dr Tt
Species

Figure 4-11). The assemblage significantly increased in pyrrolizidine alkaloid content in May, decreased significantly in June, decreased significantly once again in July and remained uniformly low through September (Dunn's Multiple Comparison Test, $p < 0.05$; Figure 4-11).. Pyrrolizidine alkaloid concentration significantly increased in October and remained high (as high as in April or May) until ithomiines disappear after December.

Interactions Between Species and Season

Seasonal variation between species. Given the importance of season to both ithomiine abundance and pyrrolizidine alkaloid concentration, I reexamined species by month. The only month in which common species distinctly differed from one another occurred between I. heraldica, I. xenos, and P. notilla in July (Table 4-3). Other months sometimes showed statistical differences between groups of ithomiine species. For these, I. xenos was consistently highest or in the highest group, while P. notilla and H. euclea always clustered in the lowest group. P. fulvescens and I. heraldica varied in their groupings for these months. There were several months in which no species differences were found. Species did not differ significantly in concentration for April (3 spp.), May (2 spp.), August (4 spp.), or December (3 spp.). This represents 4 of 9 months (44% of months with samples).

Figure 4-9. Frequency distributions of male (n = 608) and female (n = 508) ithomiines for total amount of pyrrolizidine alkaloid concentration (% dry wt.). The distributions do not differ between sexes (MW U test, p = 0.1146).

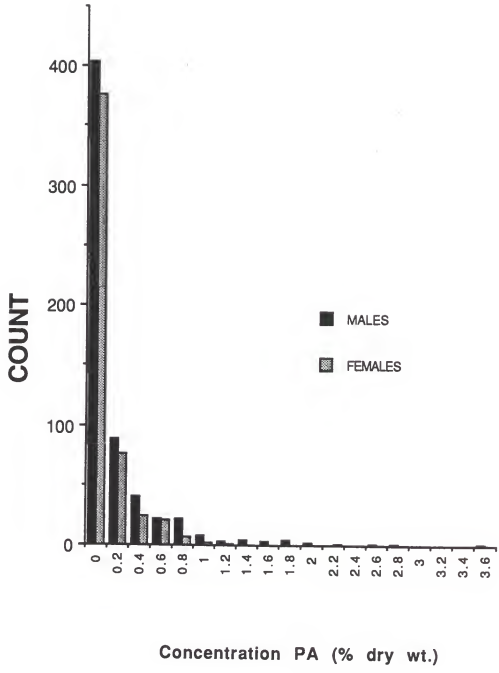

COUNT
400
300
200
100
0
MALES
FEMALES
0
0.2
0.4
0.6
0.8
1
1.2
1.4
1.6
1.8
2
2.2
2.4
2.6
2.8
3
3.2
3.4
3.6
Concentration PA (% dry wt.)

Figure 4-10. Concentration of pyrrolizidine alkaloids (% dry wt.) for ithomiines of different ages. Age is estimated as wing wear. Wing wear categories are combined for 20 tawny ithomiine species collected in one year in Monteverde, Costa Rica. F = fresh, I = intermediate, W = worn, with + and - representing fresher or more worn appearance for each category. Pyrrolizidine alkaloid concentration did not differ between wear categories (KW ANOVA, $p = 0.3596$). Sample sizes are: F+ = 77, F = 270, F- = 170, I+ = 115, I = 288, I- = 100, W+ = 30, W = 64, W- = 2. Descriptive statistics are represented in graphic format by the "box and whiskers" method of Tukey (1977).

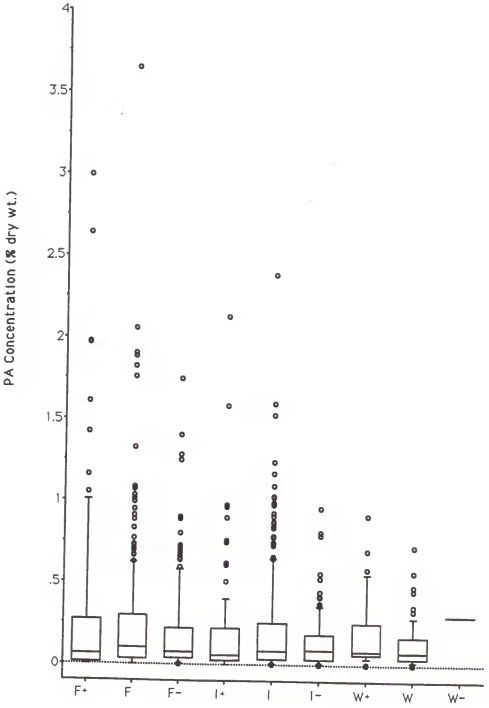
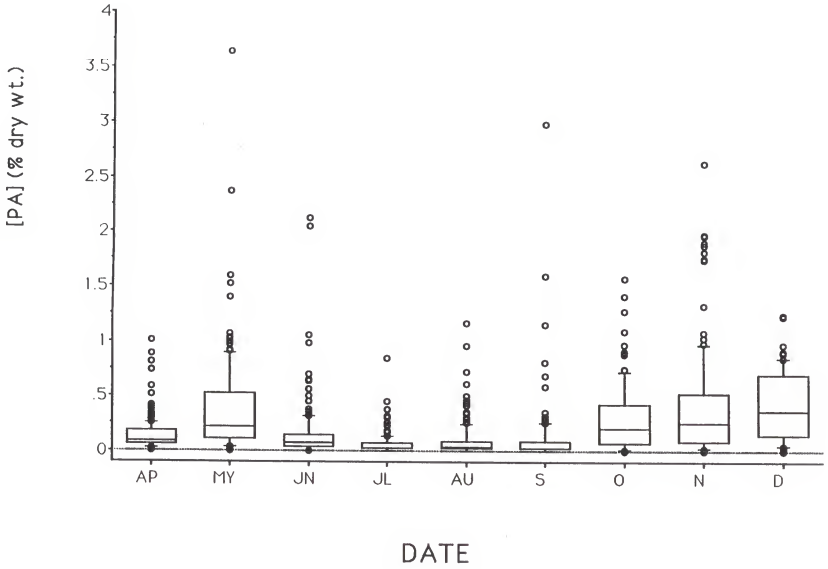

PA Concentration (% dry wt.)
4
3.5
3
2.5
2
1.5
1
.5
0
F+
F
F-
I+
I
I-
W+
W
W-
WING WEAR

Figure 4-11. Seasonal changes in the concentration of pyrrolizidine alkaloid (% dry wt.) for the tawny ithomiine assemblage of Monteverde, Costa Rica in 1987. Medians and other descriptive statistics are given each month for all tawny ithomiines captured. Monthly medians were different (KW ANOVA, $p = 0.0001$). Months were compared using mean rank (Dunn's test, $p < 0.05$) and showed the following pattern:

	AP	MY	JN	JL	AU	S	O	N	D
sample size	170	135	146	135	139	111	95	127	58
Dunn's test	BC	A	CD	E	DE	E	AB	A	A


[PA] (% dry wt.)
4
3.5
3
2.5
2
1.5
1
.5
0
AP
MY
JN
JL
AU
S
O
N
D
DATE

Seasonal variation within species. All species showed the same basic seasonal pattern: low pyrrolizidine alkaloid concentrations in the months of July, August, and September (Figure 4-12). I. heraldica, the only species abundant enough to test all months, significantly decreased in pyrrolizidine alkaloid concentration during July, August, and September, increasing in October to previous levels. The removal of I. heraldica did not alter the trends found for all species combined, suggesting the decrease in pyrrolizidine alkaloids for July, August, and September was not solely the result of a predominant species.

Additional Interactions between Species, Sex, Age, and Season

Between sexes by species by month. Within species for a given month, males showed higher median pyrrolizidine alkaloid levels than females in 72% of cases (Table 4-4). Differences were tested when each sex for a given species was represented by 10 or more individuals that month. Sexes differed in concentration of pyrrolizidine alkaloids in two of the 14 cases tested: I heraldica in April (M > F) and P fulvescens in May (F > M). The conclusion, then, is that sexes do not differ in most cases, and when different the trend is not consistent.

Age by species by month. Comparisons between fresh, intermediate, and worn individuals was only possible for I. heraldica in June. The groups were not different from one

Figure 4-12. Seasonal variation in pyrrolizidine alkaloid concentration (% dry wt.) for common ithomiine species. Medians for common species are reported each month. Statistical differences and sample sizes for common species are given in Table 4-3.

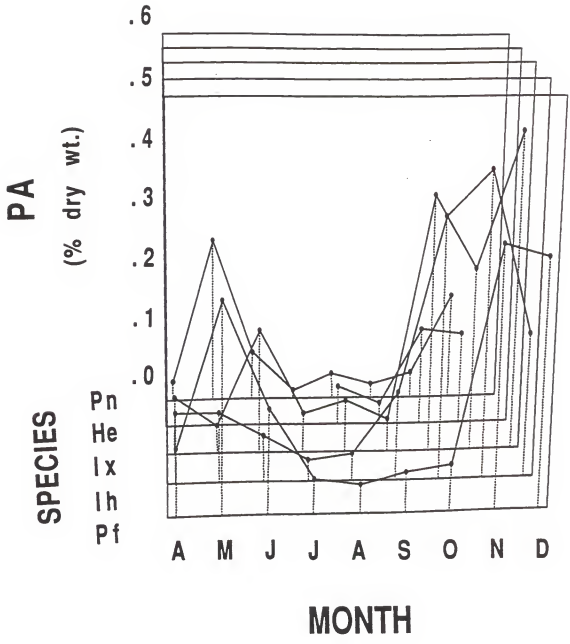

PA
(% dry wt.)
.6
.5
.4
.3
.2
.1
.0
SPECIES
Pn
He
Ix
Ih
Pf
A
M
J
J
A
S
O
N
D
MONTH

Table 4-3. Monthly patterns of pyrrolizidine alkaloid (PA) content between species measured as concentration (% dry wt. as PA per individual). Only abundant species (n > 10) were tested by Kruskal Wallace ANOVA (KW) and subsequently by the Dunn Multiple Comparisons Test (at $p < 0.05$, Zar, 1982). If only two groups are present the KW ANOVA reduces to the Mann Whitney U Test. The KW ANOVA assumes similar distribution shape and tests medians. Dunn's test examines differences between mean ranks and compensates for unequal sample size. Therefore, these tests sometimes give different results. Common species included I. heraldica (I.h.), I. xenos (I.x.), P. fulvescens (P.f.), P. notilla, and H. euclea (H.eu.).

	Median PA Concentration per Species					KW
Month	I.h.	I.x.	P.f.	P.n.	H.eu	p value
April						
Median	0.10	0.08	0.09	0.02	-	0.0002
Number	(47)	(28)	(69)	(4)	(0)	
Dunn's	A	A	A	-	-	
May						
Median	0.10	0.03	0.32	0.27	-	0.0001
Number	(39)	(3)	(69)	(5)	(0)	
Dunn's	A	-	A	-	-	
June						
Median	0.06	0.19	.15	.07	-	0.0193
Number	(61)	(26)	(3)	(21)	(0)	
Dunn's	B	A	-	AB	-	
July						
Median	0.02	0.05	0.04	0.00	-	0.0001
Number	(38)	(39)	(1)	(32)	(0)	
Dunn's	B	A	-	C	-	
August						
Median	0.03	0.07	0.03	0.03	0.05	0.0386
Number	(37)	(58)	(7)	(17)	(10)	
Dunn's	A	A	-	A	A	
September						
Median	0.13	0.04	0.05	0.01	0.02	0.0001
Number	(29)	(13)	(3)	(13)	(43)	
Dunn's	A	AB	-	C	BC	

Table 4-3--continued

October						
Median	0.41	0.41	0.06	0.03	0.15	0.0006
Number	(20)	(2)	(2)	(12)	(50)	
Dunn's	A	-	-	B	B	
November						
Median	0.48	0.29	0.40	0.17	0.14	0.0016
Number	(34)	(41)	(2)	(19)	(30)	
Dunn's	A	AB	-	B	B	
December						
Median	0.22	0.51	0.38	-	-	0.0335
Number	(15)	(37)	(6)	(0)	(0)	
Dunn's	A	A	-	-	-	

another (Kruskal Wallace test, $p = 0.8178$). For other species and months, comparing fresh and intermediate individuals similarly showed no trends. In most cases, the categories did not differ statistically (Mann Whitney U test, $p < 0.05$). Differences were found in 2 of 18 cases. Further, these two cases differed in the direction of the trend.

Ithomiine Abundance, Climate, Flowering Phenology and Pyrrolizidine Alkaloid Content

Peak ithomiine abundance from June to November corresponded to a similar peak in rainfall for these same months (Figures 4-1 and 4-2). In all, though, ithomiine abundance did not correlate with rainfall (Spearman Rank Correlation for total rainfall and total ithomiine abundance, $r = 0.03$, $p = 0.93$, $n = 9$ months). At the same time, ithomiine abundance was negatively correlated with concentration of pyrrolizidine alkaloids (SR on total amount and mean ranks, $r = -0.70$, $p = 0.048$, $n = 9$ months). July, August, and September were both the months of greatest ithomiine abundance and lowest median pyrrolizidine alkaloid concentrations.

A monthly census of pyrrolizidine alkaloid-producing plants showed a distinct period of flower availability (Figure 4-13). Two pyrrolizidine-alkaloid-producing plant species dominated the community and account for greater than 90% of the flowers counted throughout the period of ithomiine

Table 4-4. Ithomiine species and their pyrrolizidine alkaloid concentration each month by sex. Monthly medians are presented for all species represented by ten or more males and ten or more females in that month. Medians between sexes each month were tested with a Mann Whitney U test.

	Median [PA] (% dry wt.) by Month								
SPECIES	AP	MY	JN	JL	AU	S	O	N	D
I.h.									
Males	0.23	0.17	0.11	0.03	0.03	0.19	0.98	0.80	0.39
(N)	(26)	(19)	(43)	(26)	(20)	(21)	(15)	(28)	(9)
Females	0.13	0.14	0.07	0.02	0.05	0.07	0.36	0.44	0.28
(N)	(21)	(20)	(18)	(12)	(17)	(8)	(5)	(6)	(6)
MW U p =	0.01	0.80	0.31	0.22	0.74	-	-	-	-
I.x.									
Males	0.21	0.16	0.47	0.10	0.09	0.24	1.55	0.45	0.83
(N)	(22)	(1)	(21)	(20)	(32)	(2)	(1)	(17)	(19)
Females	0.09	0.04	0.18	0.06	0.08	0.06	0.39	0.37	0.58
(N)	(6)	(2)	(5)	(19)	(26)	(11)	(1)	(24)	(18)
MW U p =	-	-	-	0.06	0.53	-	-	0.85	0.09
P.f.									
Males	0.10	0.34	-	-	0.0	-	0.09	0.95	0.56
(N)	(44)	(46)	(0)	(0)	(1)	(0)	(2)	(1)	(2)
Females	0.18	0.85	0.20	0.05	0.06	0.07	-	0.17	0.53
(N)	(20)	(32)	(3)	(1)	(6)	(3)	(0)	(1)	(4)
MW U p =	0.13	0.00	-	-	-	-	-	-	-
Males	-	0.79	0.19	0.00	0.00	0.01	0.05	0.23	-
(N)	(0)	(1)	(9)	(20)	(7)	(8)	(9)	(13)	(0)
Females	0.15	0.25	0.13	0.00	0.05	0.01	0.03	0.25	-
(N)	(4)	(4)	(12)	(12)	(10)	(5)	(3)	(6)	(0)
MW U p =	-	-	-	1.00	-	-	-	-	-

Table 4-4--continued

	Median [PA] (% dry wt.) by Month								
SPECIES	AP	MY	JN	JL	AU	S	O	N	D
P.n.									
H.eu.									
Males	-	-	-	-	0.05	0.03	0.21	0.12	-
(N)	(0)	(0)	(0)	(0)	(2)	(15)	(20)	(6)	(0)
Females	-	-	-	-	0.07	0.02	0.21	0.25	-
(N)	(0)	(0)	(0)	(0)	(8)	(27)	(30)	(24)	(0)
MW U p =	-	-	-	-	-	0.32	0.46	-	-

abundance: Ageratum petiolatum and Ageratum microcarpum (Asteracaea). Ithomiines were seen at flowers of both species but were never spotted on dried plants or when flowers were not present. Koanophyllon hylonoma (Asteraceae) was also abundant in January and February but no ithomiines were seen visiting the flowers.

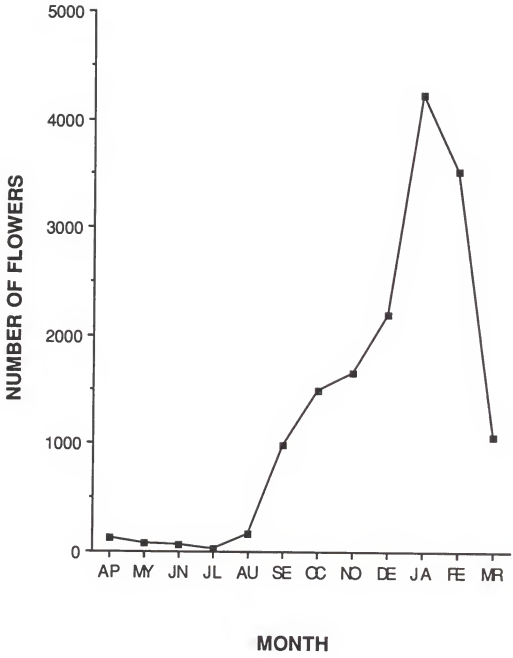
Flowering was initially low, increased abruptly in September and continued to increase into January (Figure 4-13). Neither ithomiine abundance ($r = -0.42$, $p = 0.239$) nor level of chemical protection ($r = 0.57$, $p = 0.109$) coincided significantly with flowering phenology. Nonetheless, the period of greatest ithomiine abundance and lowest pyrrolizidine alkaloid content occurred at a time when few plants producing these compounds were in flower. Moreover, ithomiine pyrrolizidine alkaloid levels increase in September and continue to increase, tracking the same pattern of flowering phenology asynchronously.

Ecological Relevance of Pyrrolizidine Alkaloid Variation

Threshold estimates

Three Blue-crowned Motmots (M. momota) ate varying numbers of P. fulvescens. In previous experiments, these birds ate more than 20 similarly-sized butterflies daily in the same amount of time (Chapter 3) so satiation was unlikely. Chemical analysis of the butterflies that were rejected showed that median concentrations differed between

Figure 4-13. Flowering phenology of plants in genera reported to contain pyrrolizidine alkaloids for Monteverde, Costa Rica 1987. Flower numbers were estimated along 3 km roadside and five 5 x 5 m tracts of pasture each month (see text).


5000
4000
3000
2000
1000
0
NUMBER OF FLOWERS
AP MY JN JL AU SE OC NO DE JA FE MR
MONTH

the three birds and a control group of 60 P. fulvescens not offered to birds (KW ANOVA, p = 0.048).

A taste rejection threshold was calculated for each bird. I subtracted the number of observations in 20 concentration categories between control P. fulvescens and those rejected by a given bird. For example, the control group showed 13 P. fulvescens for the lowest concentration interval of 0 to 0.15% dry wt., while Bird 1 did not eat 5 P. fulvescens in this category. The residual amount here is positive (since the control had more individuals in this interval than the bird left behind). Therefore, I conclude that the bird ate 8 P. fulvescens in this group. The remaining 5 were rejected for unexplained reasons, but most ithomiines in this group were palatable.

Thresholds were then determined graphically (Figure 4-14). The threshold was determined as the lowest value at which positive residuals no longer occurred (the concentration category where the control group of butterflies were no longer over-represented). For all three birds, this occurred at a concentration near 0.30% dry wt. The large positive residuals support the implicit assumption that motmots selectively ate P. fulvescens with low pyrrolizidine alkaloid levels.

Seasonal Pattern of Unpalatability

The proportion of subthreshold ithomiines depended upon month, and at times reached greater than 90%, e.g., April,

June, July, August and September (Figure 4-15). In May, October and November below-threshold ithomiines were closer to 60% of ithomiines collected. In only one month were suprathreshold individuals more abundant, some 55% in December.

Species Pattern of Unpalatability

Species differed in their degree of protection, as well (Figure 4-15). For instance, in May P. fulvescens had a majority of suprathreshold individuals while I. heraldica, I. xenos, and P. notilla were dominated by subthreshold individuals. A similar pattern emerged for I. xenos in June. P. notilla and H. euclea, unlike other species, were always represented by predominantly subthreshold individuals.

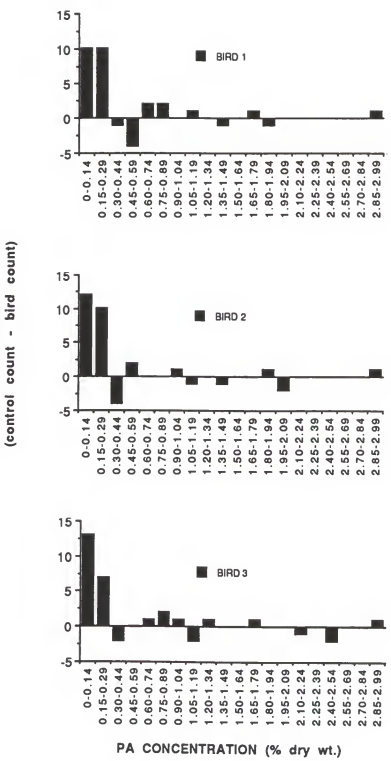
Subthreshold Individuals, Chemical Defence and Mimicry

Using the automimicry model of Brower et al. (1970), I substituted values of m and k' from this study for a range of likely n. In this way, I calculated predation risk for all ithomiines combined in different months, and then between species for months where species differed in pyrrolizidine alkaloid content. Sex and age were not tested with the model as these groups showed no consistent trends in variable defense.

Seasonal Predation Risk

Values of m used were the previous estimates of absolute population size (Figure 4-2). Values of k' used were the

Figure 4-14. Palatability threshold (% dry wt. of pyrrolizidine alkaloid per individual) determined from residual counts of a single control group of 60 P. fulvescens and similar samples offered in lots of 10 each day to Blue-crowned Motmots (Momotus momota: Momotidae). P. fulvescens eaten by birds are absent. Residuals are calculated as the difference between control ithomiines and those rejected by each bird. Hence, a positive number suggests ithomiines with these levels of pyrrolizidine alkaloids were palatable. No difference represents no differential treatment. Negative values represent an initial sampling bias.


RESIDUALS
(control count - bird count)
BIRD 1
BIRD 2
BIRD 3
15
10
5
0
-5
0-0.14
0.15-0.29
0.30-0.44
0.45-0.59
0.60-0.74
0.75-0.89
0.90-1.04
1.05-1.19
1.20-1.34
1.35-1.49
1.50-1.64
1.65-1.79
1.80-1.94
1.95-2.09
2.10-2.24
2.25-2.39
2.40-2.54
2.55-2.69
2.70-2.84
2.85-2.99
PA CONCENTRATION (% dry wt.)

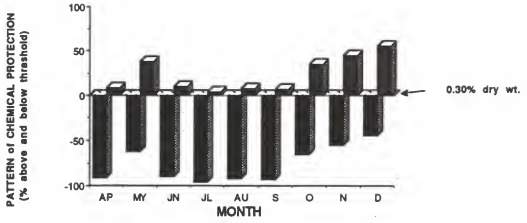
proportion of protected individuals, or the suprathreshold individuals of Figure 4-15. Values of n were not determined experimentally, so I used a range from 0 to 100. It is likely that a single bird would eat about 20 ithomiines per day (Chapters 2 and 3). I saw no more than two motmots at any one time in the assemblage, but five individuals is within possible limits.

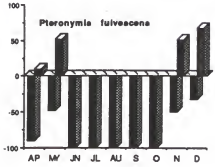
Monthly Patterns of Predation Risk

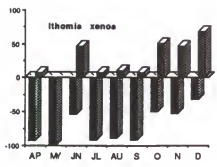
Abundance-related patterns. Predation risk for the average ithomiine varied from month to month. In the absence of chemical defense, the model predicted that the average ithomiine was least likely to suffer predation when ithomiine abundance was high (j_o, Figure 4-16). As previously mentioned, this occurred in July, August and September.

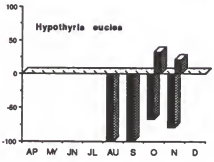
Variable defense and mimicry. Predation risk decreased further in all months if some ithomiines were chemically defended ($j_{k'}$; Figure 4-16). The magnitude of the difference (ΔJ) is a measure of mimetic advantage (Figure 4-17). The mimetic advantage was low for most months, a result of both large population size and a large percentage of unprotected individuals. July, August, and September are the months in which mimicry is least effective. April, May, June, November, and October offer slightly better conditions for mimicry. It is in December that mimicry is most advantageous, however. December has few ithomiines and most of these are above threshold. For a single motmot in

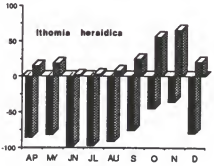
Figure 4-15. Proportion of unpalatable tawny ithomiines in Monteverde, Costa Rica in 1987. Values are based upon the percentage of supra- and subthreshold individuals from the threshold reported in Figure 4-14 (and text) and concentration (% dry wt.). Sample sizes are reported in Figure 4-4.

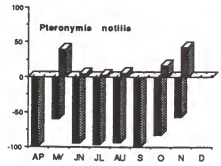

PATTERN of CHEMICAL PROTECTION
(% above and below threshold)
100
50
0
-50
-100
AP MY JN JL AU S O N D
MONTH
0.30% dry wt.


Pteronymia fulveacena
100
50
0
-50
-100
AP MY JN JL AU S O N D


Ithomia xenos
100
50
0
-50
-100
AP MY JN JL AU S O N D


Hypothyris eucles
100
50
0
-50
-100
AP MY JN JL AU S O N D


Ithomia heraldica
100
50
0
-50
-100
AP MY JN JL AU S O N D


Pteronymia notilia
100
50
0
-50
-100
AP MY JN JL AU S O N D

December hungry enough to consume 20 ithomiines, the average ithomiine is 10% less likely to be eaten as a result of chemical defense and mimicry. Compare this to other months when the same bird is likely to consume many more ithomiines before encountering an unpalatable one. In July, August or September, the average ithomiine gains less than 0.01% from mimicry.

Interaction of abundance, variable defense and mimicry. During months of lowered chemical protection, the population size is so large that the likelihood of any individual being sampled is still small. Even in the absence of chemical defense, the probability of survival (J_o) is much greater in August than in December (J_o = 0.889 for December, J_o = 0.989 for August). Adding mimetic advantage differentially changes survivorship, but only enough to make the probabilities of survival nearly equal for the average ithomiine in these two months ($J_{k'}$ = 0.989 in December vs. $J_{k'}$ = 0.994 in August). The probability of surviving was, by these estimates, uniformly high for all months (Figure 4-16).

Despite the ameliorating effects of abundance, months differed by as much as 0.02% in the likelihood of ithomiine survival. July was the month of greatest risk, followed by April and December (Figure 4-16, insert). Other months were above 99% survivorship. July was a month of intermediate abundance but the lowest proportion of protected individuals. April had fewer ithomiines, but more with protection.

Figure 4-16. Changing probability of survival for tawny ithomiines present in different months. Probability of survival is shown for a single predator or group of predsators that could eat between 0 and 100 ithomiines. Abundance and proportion of protected ithomiines were used to calculate survival with the model of Brower et al. (1970). Each line represents a different month. Abbreviations for months are listed in order, corresponding to the line that represents them. Abbreviations listed side by side denote months where lines completely overlap. In the absence of chemical defense, months differed greatly in their predation risk to the average ithomiine (Jo). This difference is based solely upon abundance. With variable defense, survival improved so that months were more similar. Differences to survival between months were small with variable defense (insert), but show that months changed in their ranking caused by monthly changes in chemical protection.

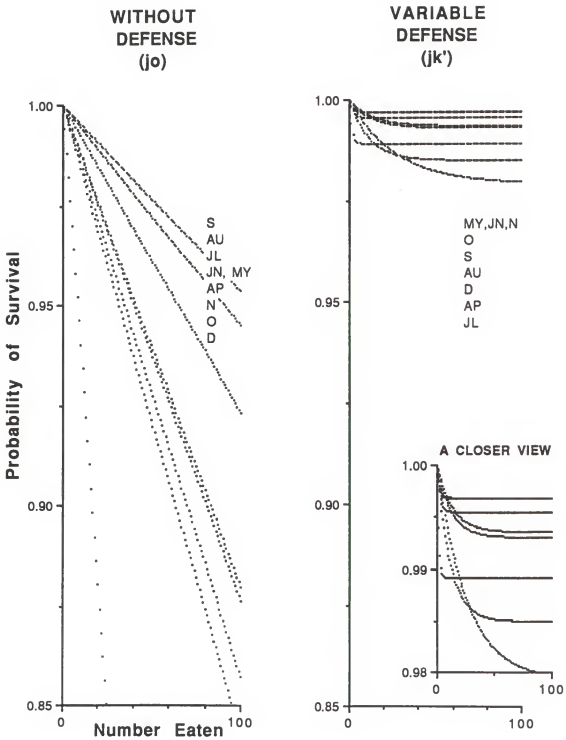
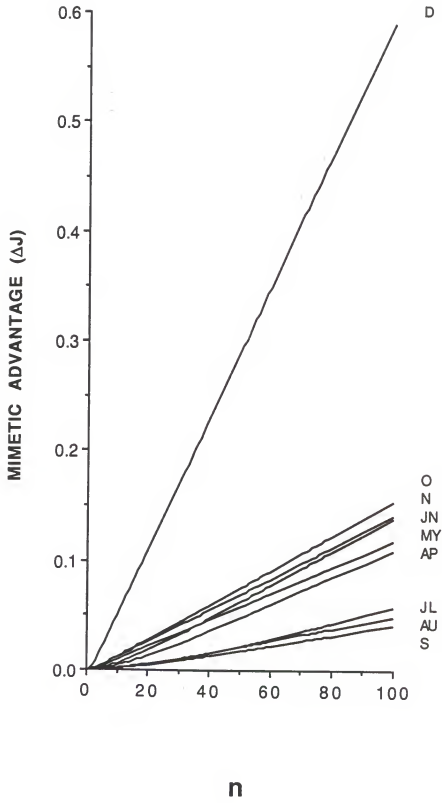

WITHOUT DEFENSE (jo)
VARIABLE DEFENSE (jk')
Probability of Survival
1.00
0.95
0.90
0.85
0
100
Number Eaten
S
AU
JL
JN, MY
AP
N
O
D
MY,JN,N
O
S
AU
D
AP
JL
A CLOSER VIEW
1.00
0.99
0.98
0
100

Figure 4-17. Mimetic advantage for tawny ithomiines by month. Values are calculated using automimicry models of Brower et al. (1970) assuming all tawny ithomiines are viewed by a predator as a single morph. Values of population size (m) are those from Figure 4-2, the proportion of unpalatable individuals (k') are from Figure 4-15. The number of ithomiines a predator may consume in a unit of time (n) are represented here as a range from 0 to 100. The mimetic advantage (ΔJ) is the additional likelihood of an individual escaping predation as a result of mimicry in a population (here an assemblage) with variable palatability. There is a mimetic advantage in all cases, but it is much greater in December as a result of low population size and a greater proportion of unpalatable individuals.


MIMETIC ADVANTAGE (ΔJ)
0.6
0.5
0.4
0.3
0.2
0.1
0.0
0
20
40
60
80
100
n
D
O
N
JN
MY
AP
JL
AU
S

December was the month of both lowest abundance and the greatest proportion of protected individuals.

Patterns of Survival Between Species

Abundance-related patterns. As with ithomiines in general, species were differentially protected solely on the basis of their relative abundance (Table 4-5). A case in point is the month of April. Species in this month did not differ in pyrrolizidine alkaloid content according to the Dunn's test. As a result, the proportion of unpalatable individuals did not vary between species. The species did differ in their abundance, however. P. fulvescens had a population size of 470, compared to 202 I. heraldica, 73 I. xenos, and 8 P. notilla. If motmots distinguished species without error (if they were not mimics), the model predicted the probability of survival, jk', would increase with abundance, despite similar levels of protection. The model also showed that, when variable defense was equal between species, the advantage of Müllerian mimicry (ΔJk') was greatest for species that were rarest. This "selfish herd effect" occurred to varying degrees in all cases where species showed similar proportions of unpalatable individuals but different population sizes.

In all, for six cases of large gains from automimicry ($\Delta J > 0.1$; Table 4-5), 3 cases resulted from a high proportion of unpalatable individuals, 1 case was largely the outcome of low population size, and 2 cases were a

combination of both. For 3 cases of low gains in automimicry ($\Delta J < 0.0001$), all may be attributed to few protected individuals. Müllerian mimicry benefitted individuals of species that had relatively small population sizes (all 3 cases with $\Delta Jk' > 0.1$). Conversely, of the 3 situations where Müllerian mimicry conferred a small advantage ($\Delta Jk' < 0.005$), all were attributed to species with a high proportion of unpalatable individuals.

Variable Defense and Mimetic Advantage

In December, each species represented a different condition. I. xenos was abundant and the majority of individuals were unpalatable. I. heraldica, on the other hand, was of intermediate abundance and the incidence of unpalatability for a predator specializing on this species was low. Finally, P. fulvescens was uncommon but, like I. xenos, was represented by a large proportion of unpalatable individuals. Assuming a single predator that could eat 20 ithomiines in a single day, the likelihood of surviving the day varied drastically in the absence of both mimicry and chemical defense (J_O): the majority of I. xenos would survive, about half of I. heraldica, and all P. fulvescens would be consumed. Add variable chemical defense and protection was nearly the same for the three species. Any I. xenos escaped predation by hiding in a large population, but increased its chance of escape an average of 17%, because any prior experience was, by chance, unpleasant for the predator.

Table 4-5. Likelihood a single tawny ithomiine would survive a bout of predation. I use a model by Brower et al. (1970) to assess predation risk to individual ithomiine butterflies from a single predator able to eat 20 butterflies. I measure probability of survival for individuals of abundant species in all months. I determine survival when all members of a species lack defense (Jo) and when some proportion are protected (Jk'). Abundance and proportion of individuals protected were measured for each species for each month. I then calculate the additional likelihood of survival resulting from automimicry and variable defense (ΔJ = Jo - Jk'). Finally, I compare values of Jk' for species and all species (rare and common) in the ithomiine assemblage to calculate the increased survivorship by Müllerian mimicry ($\Delta Jk'$ = Jk'assemblage - Jk' species).

	Probability of Survival			
	No Defense (Jo)	Variable Defense (Jk')	Mimetic Gain Auto (ΔJ)	Müllerian ($\Delta Jk'$)
April				
I. heraldica	0.903	0.947	0.044	0.041
I. xenos	0.730	0.811	0.082	0.176
P. fulvescens	0.957	0.980	0.022	0.008
May				
I. heraldica	0.921	0.978	0.057	0.018
P. fulvescens	0.960	0.996	0.036	0.001
June				
I. heraldica	0.928	0.938	0.010	0.049
I. xenos	0.831	0.982	0.150	0.005
P. notilla	0.811	0.876	0.065	0.110
July				
I. heraldica	0.952	0.962	0.010	0.027
I. xenos	0.949	0.973	0.025	0.016
P. notilla	0.927	0.945	0.181	0.044
August				
H. euclea	0.900	0.900	0.000	0.094
I. heraldica	0.956	0.978	0.022	0.016
I. xenos	0.967	0.985	0.019	0.009
P. notilla	0.926	0.956	0.030	0.038

Table 4-5--continued

	Probability of Survival			
	No Defense (J_o)	Variable Defense (J_k')	Mimetic Gain Auto (ΔJ)	Müllerian ($\Delta J_k'$)
Sept.				
H. euclea	0.977	0.977	0.000	0.018
I. heraldica	0.964	0.992	0.028	0.002
I. xenos	0.930	0.964	0.034	0.031
P. notilla	0.930	0.930	0.000	0.064
Oct.				
H. euclea	0.940	0.991	0.050	0.005
I. heraldica	0.851	0.986	0.136	0.009
P. notilla	0.750	0.927	0.177	0.068
Nov.				
H. euclea	0.881	0.970	0.090	0.026
I. heraldica	0.894	0.992	0.098	0.005
I. xenos	0.911	0.990	0.080	0.006
P. notilla	0.796	0.976	0.180	0.021
Dec.				
I. heraldica	0.535	0.885	0.350	0.104
I. xenos	0.813	0.986	0.174	0.003

P. fulvescens individuals escaped predation by chemical defense only, with a mimetic advantage of 0.91. Finally, I. heraldica showed an intermediate mimetic advantage of 0.35. In the end, an individual in this population suffered the greatest risk of predation of the three species.

If the species joined the other two in a mimetic complex, the survivorship increased further, even in the absence of chemical defense (ΔJ_0). A P. fulvescens increased its probability of survival from 0 to 0.88 by increasing its effective population size in the eyes of the predator. This allowed P. fulvescens to hide, or restricted the predator from specializing on it. In lesser respects, this was true for members of all species, but only when predators did not respond numerically. Above this "selfish herd" effect was the additional advantage of chemical defense combined with mimicry ($\Delta J_{k'}$). Though I. heraldica and P. fulvescens gained most, individuals of all three species increased survivorship as a result.

Discussion

Chemical defense in ithomiine butterflies is contingent upon acquisition and adequate storage of pyrrolizidine alkaloids. Ithomiines vary in pyrrolizidine content (Brown, 1984, 1985). Identifying the sources and outcome of variable chemical defense for these butterflies is fundamental to understanding ithomiine ecology and the evolution of their

Table 4-6. Likelihood of an average ithomiine surviving an attack and its mimetic advantage for December. Values for survivorship in the absence of chemical defense (J_0), with a proportion, k', chemically protected ($J_{k'}$) and the advantage of defensive chemistry ($\Delta J = J_0 - J_{k'}$) are calculated from a model by Brower et al. (1970). Population sizes (m) are from estimates in Figure 4-3. The proportion of individuals protected is taken from Figure 4-15. The advantage of being mimetic within species ("automimetic," Brower et al., 1967) is given as ΔJ for each species. The value of belonging to a mimicry complex is ΔJ_0 ($J_{0\ TOTAL} - J_{0\ species\ i}$) in the absence of chemical defense. An advantage in this case is a swamping, or "selfish herd" effect (Hamilton, 1964). With variable chemical defense, the mimetic advantage to a member of a mimicry complex is $\Delta J_{k'}$ (Jk'assemblage - Jk'species). An advantage in this case would be that in addition to ΔJ_0. Both assume that members of a mimicry complex are seen as a single morph by a predator that would eat 20 individuals in the absence of chemical defense. Once encountered, an unpalatable is encountered foraging ends on members of that species or species complex.

Species	m	k'	J_0	Jk'	ΔJ	ΔJ_0	$\Delta J_{k'}$
I. xenos	106	0.68	0.81	0.99	0.17	0.07	0.003
I. heraldica	43	0.20	0.53	0.88	0.35	0.35	0.104
P. fulvescens	17	0.67	0.00	0.91	0.91	0.88	0.090
TOTAL	166	0.55	0.88	0.99	0.11	-	-

extensive mimicry. Here, I present the first observations necessary to evaluate the sources and outcome of variable defense in ithomiines: pyrrolizidine alkaloid content for all similarly-colored species in a single geographical location for an entire season.

Patterns of Pyrrolizidine Alkaloid Variation in Ithomiines

Season

Of all factors explored, month was the principal determinant of ithomiine pyrrolizidine alkaloid concentration. Further, the seasonal variation in ithomiine pyrrolizidine alkaloid content coincided with a seasonal change in pyrrolizidine alkaloid sources. In Monteverde, the majority of pyrrolizidine alkaloid plants produce flowers late in the wet season, with ithomiine pyrrolizidine alkaloid content trailing.

Species

Species effects were found, but differences within and between species varied from month to month in no predictable way. If species-level ecological chemistry is the source of the species patterns found here, the mechanisms are not obvious. The differential use of pyrrolizidine alkaloids by species necessary to explain species-level patterns must be subject to seasonal factors, as well.

Sex and Age

Sexes and ages did not differ in pyrrolizidine alkaloid concentration. This means that the net effects of sex differences in the way pyrrolizidine alkaloids are obtained and lost are the same. If males lose more alkaloids to females in spermatophores and pheromone production, they compensate by quick renewal of pyrrolizidines from plant sources. Age similarities suggest that adults acquire pyrrolizidines rapidly post-emergence, or that older ithomiines use up pyrrolizidines rapidly so that both old and new adults face the same problems of pyrrolizidine alkaloid acquisition.

Ecological Outcomes of Ithomiine Pyrrolizidine Alkaloid Variation

In Monteverde, the majority of ithomiines are palatable when ithomiine abundance is greatest. Under these conditions, ithomiine abundance and chemical protection interact to produce largely similar risks of predation throughout the year. In other words, for tawny ithomiines in Monteverde, the seasonal decrease in pyrrolizidine alkaloid content may not increase risk of predation. Theoretically, the greatest risk comes with low abundance and low protection. Similarly, greatest protection occurs at high density and high abundance. These conditions did not occur in Monteverde. For this assemblage, ithomiines were either abundant with low pyrrolizidine alkaloid stores or of lower

abundance with relatively high numbers of protected individuals

In short, the model proposes that rare groups and those least-protected will gain most from mimicry. Even in cases where species, sexes, and ages did not differ in pyrrolizidine alkaloid concentration, relative abundance often differed markedly. Thus, common species did not differ in pyrrolizidine alkaloid concentration in April (Table 4-3), but differences in relative abundance (Figure 4-3) suggest that, while members of all species benefit, I. xenos benefit most from both automimicry and Müllerian mimicry because of their relatively low abundance (see also Table 4-4). Sexes were male-biased in three months, as well. As a result, despite similar protection, females gained more from mimicry than males in these months.

The assumptions for use of the model here are: (1) palatability is determined by suprathreshold amounts of pyrrolizidine alkaloids, (2) birds exhibit associated negative conditioning with single trial learning, and (3) that birds do not respond numerically to ithomiine abundance. Threshold-related palatability of pyrrolizidine alkaloids is discussed in Chapter 2. Single trial conditioned avoidance has been demonstrated for cardenolides in the monarch (Danaus plexippus, Brower and Fink, 1985; Huheey, 1988). A numerical response on the part of predators is likely, however. Although ithomiine abundance increases rather abruptly, high abundance continues for four months. Large numbers of

predators tracking ithomiine abundance would put ithomiines at greater risk during the three of four months of lowest pyrrolizidine alkaloid content. If relative predator and ithomiine abundances remained constant (so that n/m was a constant), mimetic advantage would decline with lower numbers of protected butterflies. In this case, abundance would not ameliorate the increased predation pressure that comes with decreased chemical defense (see Chapter 5).

The Cost of Defense and Variable Chemical Defense

There may be a cost to defense as well as the benefits from lower predation (Bowers, 1988). Secondary compounds used in defense may require additional foraging, metabolism, and may cause low level toxicity to the insect incorporating them. Evidence of such costs have been measured only for defenses acquired as larvae in danaines and nymphalines (Bowers, 1988). Growth rate and adult size are usually not influenced by amount or presence of defensive compounds and the presence of secondary compounds may even enhance growth.

There are no data to support a cost to pyrrolizidine alkaloid defense in ithomiines. If such costs occurred, the pattern of low defense in times of high abundance might be explained by costs alone. In this case, ithomiines at high abundance may swamp predators, gain little from defense and avoid it, thereby avoiding associated costs. Costs may also differ between species. If costs are lower for I. xenos and

I. heraldica, this may explain why they are more consistently protected than P. notilla and H. euclea.

Evolution of Mimicry and Variation in Ithomiine Pyrrolizidine Alkaloid Content

Mimicry theory was devised to explain coincident coloration between species. The evolutionary outcome of the simplest case of two species has been discussed by several authors (see Huheey, 1988). When one species is uniformly protected (model) and the other is uniformly not (mimic), the result is advergent evolution (Brower and Brower, 1972) where the mimic converges on the model but the model does not. Changing the relative abundance of the two species will change the rate of evolution but not its outcome. When both species are equally and uniformly unprotected they will converge in color pattern. Relative abundance alters the outcome in favor of the most abundant morph. Relative protection alters the outcome in favor of the most unpalatable or unpleasant.

For ithomiines the predictions are more complicated. No species is uniformly protected and change both their seasonal relative palatability and abundance. The month of December provides an example (Table 4-6). Using the model of Brower et al. (1970), survival values with variable palatability are calculated for individuals of three species ($J_{k'}$). This value assumes the species do not mimic one another. The values for each species can be compared with the case when

all three species are perfect co-mimics of one another, i.e., when colors match so closely a predator cannot tell them apart (in Table 4-6, this is the TOTAL row). Note that all three species gain from mutual resemblance ($\Delta J_{k'}$), as predicted for simpler cases of Müllerian mimicry (Huheey, 1988). Therefore, all three species should converge upon a common pattern.

The relative rates of convergence will determine the outcome, as well. Whichever species is prone to greatest predation when treated separately by a predator should converge most quickly on the more protective pattern. This is measured as the magnitude of $\Delta J_{k'}$. In December, two species converge at similar rates but for different reasons. I. heraldica is abundant but the majority of its individuals are poorly protected. Conversely, the P. fulvescens is uncommon but with more unpalatable members. The species converging least rapidly is I. xenos, both abundant and with many well-protected individuals. For December, then, the evolutionary outcome of predation for ithomiines would be a color pattern that is a conglomerate of elements from all three species. In general, the pattern should be most similar to the current pattern of I. xenos

The intensity and direction of color pattern evolution by natural selection for tawny ithomiine in Monteverde is sufficiently complex that attempting a prediction of the color pattern upon which all species will eventually converge is not realistic. All species were at least partially

protected by pyrrolizidine alkaloids, and I showed here that members of all species benefit from converging to a common pattern, above and beyond abundance effects. Rare species with a high proportion of palatable individuals will converge more quickly upon common, well-protected species than common, well-protected species will converge upon them. The ecological dynamics are so diverse for ithomiines in Monteverde, however, that evolution may never stabilize to a single pattern. Instead, an unpredictable series of compromises will result. An end pattern will never be reached, in effect, because species vary in relative abundance, are not always together, and ecological conditions are unstable. At the same time, selection will favor closer resemblances so that, over time, similarities in color pattern between species should increase. On top of these considerations, differential costs of chemical defense (see above) could further alter the fitness of individuals involved in the mimicry.

There is a long-standing hypothesis in mimicry theory that there is no selective pressure for Müllerian mimics to closely resemble each other (Cott, 1940). It is argued that the predator gains by generalizing. In Müllerian systems with variable degrees of automimicry, this may not be. Some species are represented by individuals nearly wholly unprotected flying alongside a species with much higher proportions of unpalatable members. Certainly, a predator should specialize on I. heraldica in December given poor non-

ithomiine alternatives. This contradicts the idea of predators generalizing. In this situation, prey species that loosely resemble each other would be selected to converge. Nonetheless, the existence of variable appearance and abundance of ithomiine species, coupled with seasonal changes between species in degree of automimicry, may make closer mimetic resemblances slow to evolve.

Summary

Variation in defense compounds was monitored for all tawny ithomiines within a single assemblage in Monteverde, Costa Rica. Pyrrolizidine alkaloid content varied between species at times, but in no consistent way. Sexes did not differ, nor did ithomiines of different ages. Season had a marked effect. Pyrrolizidine alkaloid concentration decreased significantly from June to September. Concentrations increased thereafter, following the flowering period of pyrrolizidine alkaloid-containing plants. Thresholds estimated from feeding experiments with the Blue-crowned Motmot suggest that variable pyrrolizidine alkaloid concentration translates to variable palatability. Using a model by Brower (et al., 1970), I assess ithomiine survivorship under conditions of variable abundance and chemical defense. In Monteverde, abundance was highest when defenses were lowest. Under the assumptions of the model, these conditions kept risk of predation uniformly low. All

individuals benefitted from mimicry, but individuals in rare and/or less well-protected species gained most. Some species were effectively Batesian mimics at times. The evolution of mimicry represents a compromise from predation-based selection that changes direction to track wide changes in ithomiine relative abundance and variable chemistry.

CHAPTER 5
GENERAL CONCLUSIONS

"When no question of degree is introduced into the discussion nothing is clearer than the distinction between the Batesian and Müllerian factors. If, however, we take into consideration that butterflies may exist in all degrees of palatability, and that avoidance or acceptance by the predator must depend greatly upon its appetite, there is some danger that the distinctness of the evolutionary tendencies may be lost in the complexity of actual biological facts."

Fisher, 1928.

Variation in chemical defense and its resultant palatability spectrum (Brower, 1969) have placed the Batesian-Müllerian dichotomy in doubt (Huheey, 1976). Yet the distinction between Batesian and Müllerian species is fundamental to predictions of the current utility and future evolution of aposematism, chemical defense, and mimicry (Ritland and Brower, 1991). Batesian species converge upon protected species and are subject to frequency-dependent selection, where the benefits of mimicry decrease with increasing relative abundance of the Batesian species. Protected species, on the other hand, either diverge (Brower and Brower, 1972) or remain unchanged (Turner, 1987) while Batesian species converge on them. Müllerian species converge upon each other's color pattern and the benefits of mimicry increase with increasing abundance. Whether the palatability spectrum discussed and documented in Chapter 4

destroys the classic dichotomy of Batesian and Müllerian has been argued (Benson, 1977; Sheppard and Turner, 1977; Turner, 1987; Huheey, 1988). Two models define the debate (Turner, 1987; Huheey, 1988). The resolution of the debate, I believe, is three fold: (1) to explicitly incorporate prey abundance into models, (2) to cast off the typological notion of species-level protection and, instead, acknowledge variation both between and within species, and (3) examine the influence of predator foraging behavior. I have already dealt with the first two considerations. In this chapter, I reexamine the influence of prey abundance and variable defense to mimetic advantage when predator behavior changes.

The Batesian-Müllerian Continuum

The Huheey model (1976) predicts a continuum from Batesian to Müllerian. The model is based upon a memory parameter, n-1, which is the number of butterflies a predator skips after sampling an unpalatable butterfly. In a Batesian-Müllerian situation, the probability that a butterfly will be eaten is

$$P = 1/(p + nq),$$

where P is the probability that an individual will be eaten, n is the memory parameter (how many prey will be skipped after sampling an unpalatable prey item), p is the frequency

of the Batesian species, and q is the frequency of the Müllerian species. This model can be extended to Müllerian assemblages of many species:

$$P = 1/(pm + qn + ro...).$$

Here, the frequencies of species are p, q, r, etc., with memory parameters (proportional to degree of unpalatability) of m - 1, n - 1, o - 1, and so on. A Batesian species in this case has a memory parameter of 1.

The idea of a continuum from Batesian to Müllerian is based upon the change in predation risk with the addition of a more or less-protected species. If a species is extremely unpalatable, its presence, according to classic mimicry theory (Huheey, 1988), should decrease the predation risk for members of all species because more butterflies are skipped before the predator tries prey with that color pattern again. A more palatable species would elicit fewer skips (see Huheey, 1988 for examples of predators that behave in this way).

Thus, as in classical mimicry theory, the presence of a palatable species diminishes the protection for members of more unpalatable ones. However, Huheey's model contradicts classic mimicry theory with Müllerian species (when both species are protected). Instead of an increased benefit for individuals of both protected species, Huheey finds that members of the more-protected species lose as a result of the

mimicry, qualitatively similar to the case with a Batesian species. What changes is quantitative: The more palatable the mimic, the greater the loss to members of the protected species. For instance, a bird eats an individual of species A and skips the next 10 butterflies. The same bird encounters species B and skips the next 5 butterflies. If these butterflies shared the same color pattern, species A would be sampled more and species B would be sampled less. The average decision by the bird would be to skip the next 7.5 butterflies. Therefore, unlike the prediction of classical mimicry theory, one Müllerian species negatively influences another based upon its degree of unpalatability. The more palatable the species, the more classically "Batesian" its influence on color pattern evolution. Thus, species may be ranked along a Batesian-Müllerian continuum.

The Batesian-Müllerian Dichotomy

A model by Turner (1987), on the other hand, reaffirms the Batesian-Müllerian dichotomy. It is based upon the assumption that predators learn to attack palatable species and learn to avoid unpalatable ones. He uses Monte Carlo simulations based upon the following system of predator behavior. A pleasant experience increases the probability of attack and a distasteful experience decreases it. As no prey are encountered or sampled, the probability of attack returns asymptotically to 50%, which is the rate of attack on neutral

prey (neither distasteful nor palatable, these will neither decrease nor increase the probability of attack). Here, the relative palatability of model and mimic is implicitly defined by the butterfly's effect on attack probability. Members of more unpalatable species cause the predator to hesitate longer (skipping more butterflies) while a palatable butterfly increases predation.

Under these conditions, Turner (1987) shows that the Batesian and Müllerian categories are distinct, generating a sigmoid curve with a narrow inflection point for "neutral" species. The claim is based upon relative predation rates for: (1) a uniformly unpalatable species of unique color pattern (Nasty), (2) a uniformly unpalatable species of mimetic color pattern (Model), (3) a mimetic species whose degree of unpalatability changes (Mimic), and (4) a non-mimetic species whose degree of unpalatability changes (Solo).

At the extreme, when all 4 species are most unpalatable, species suffer similar rates of predation. If Mimic and Solo similarly range from most unpalatable to neutral, then Nasty, Model, and Mimic have equally low predation rates, while Solo's gradually increase. When Mimic and Solo become palatable, Model becomes more heavily preyed upon than Nasty, and Mimic is eaten less frequently than Solo. Thus, Müllerian species (both Model and Mimic unpalatable) are similarly protected while Model protects Mimic (when palatable) at its own expense. The change from mutual

benefit to parasitism is abrupt when Mimic becomes palatable, and, according to Turner (1987) supports the traditional Batesian-Müllerian dichotomy.

Continuum or Dichotomy?

Huheey's model predicts a continuum while Turner's upholds a dichotomy. A closer look reveals why. Despite their claims, the models do not contradict each other. What differs is the way in which they measure prey density and define predator foraging behavior. In the end, the models describe different conditions and offer two principal differences in their predictions: (1) the relative predation risk for Müllerian mimics of differing palatability, and (2) the abruptness of the Batesian-Müllerian continuum.

Are Müllerian Species Mutualists or Parasites?

For chemically protected species sharing similar color patterns, Huheey's model predicts that more-protected species suffer at the expense of less-protected ones. Turner and classical mimicry theory predict that all species will benefit, so long as they are somewhat unpalatable. Each compares the probability of attack when two species are together with the probability of attack when there is only one species present. The models differ, however, in how they measure prey abundance. Huheey keeps overall abundance of

butterflies constant (Sheppard and Turner, 1977; Benson, 1977). Because total population size is fixed, the addition of one species halves the population size of the formerly solitary species. Turner, on the other hand, implicitly keeps the number of individuals of each species constant.

Both models are correct. If a less well-protected species colonizes, then competes with the first and effectively halves the population size of the more-protected species (as with Huheey), the average predation risk to the more-protected species will rise. If the second species does not affect the abundance of the first, both species will benefit from the sympatry, as Turner suggests. At the very least, this debate suggests the importance of explicitly incorporating relative and absolute abundance into these mathematical models. Owen and Owen (1984) effectively alter the Huheey model to incorporate abundance and agree with the conclusions of Turner and classical mimicry theory (Huheey, 1988).

The "Knife-edged" Separation

Inhibition vs. Induction

The dichotomy of Batesian and Müllerian mimicry is supported by the model of Turner, but Huheey's model predicts a continuum from Batesian to Müllerian where the relative gains from mimicry grade from parasitic to mutualistic. Again, a closer look reveals no argument between the models.

The difference arises in the ways variable palatability translates into predator behavior. In Huheey's model, the predator skips numbers of butterflies based upon relative palatability. Therefore, a population of palatable butterflies sets the number at zero. Here, the predator eats all butterflies it encounters. Unpalatability causes the predator to skip. Predator behavior is, thus, built upon differential inhibition. Turner, however, allows both inhibition and induction. Therefore, palatable butterflies actually increase butterfly predation by the predator. The inflection point or "knife-edge" (Benson, 1977) occurs at the transition of inhibition and induction (a single point of neutrality exists between them where a butterfly neither induces not inhibits further butterfly predation). If the analysis of Turner is restricted to include only inhibition, the inflection point disappears, and the pattern resembles a continuum.

Predator Foraging Decisions

Abrupt change. The "knife-edge" is also the result of the precise rules for predator foraging decisions and butterfly replacement built into Turner's model. Turner assumes that if a predator attacks a butterfly and it is unpalatable, the likelihood of the next attack is calculated by multiplying the existing probability of attack by a constant. The constant corresponds to relative palatability. The equation implicit in this rule may be written:

$$P_{n+1} = P_n \beta^n,$$

where P_{n+1} = probability of eating the next butterfly, Pn is the initial probability of eating a butterfly [Turner sets this at 0.5 and allows the predator to "forget" so that Pn is constantly returning to 0.5 at a rate of:

$$Pn+1 = (Pn - 0.05)\ 0.98 + 0.05],$$

ß is the constant that determines degree of induction or inhibition, and n is the number of butterflies previously sampled.

The result of this foraging behavior is that more unpalatable butterflies disproportionately inhibit and highly palatable butterflies disproportionately induce predation. For example, a bird has an initial probability of attack of 0.5. If it eats a somewhat unpalatable butterfly (ß = 0.8; 1 = neutral and 0 = most unpalatable), the probability of next attack is 0.5 x 0.8 = 0.40. The next attack on a similar butterfly would further reduce attack probability to 0.5 x 0.8 x 0.8 = 0.32. If the same predator had contacted a butterfly twice as unpalatable (ß = 0.4), the probability of a future attack would be 0.5 x 0.4 = 0.20. Another of this type would decrease the attack probability by 0.5 x 0.4 x 0.4 = 0.08. Thus, a halving palatability results in a 4-fold difference in predation pressure from two encounters, an 8-fold increase from 3 encounters, and so on. The exponential portion of Turner's (1984, 1987) sigmoidal curve, therefore,

is a direct result of this predator-learning assumptions. The asymptote for Solo when palatable is, then, an artifact of sampling without replacement.

Linear continuum. The apparent Batesian-Müllerian dichotomy could just as easily be a linear continuum, even with induction, if the predator's rules change. If predators decreased the likelihood of attack using a simpler function, the result would be a straight line. For instance, the probability of attack could be written as:

$$P_{n+1} = ßPn.$$

Here, Pn is a constant, set at 0.5 (for the sake of comparing with the starting point of the previous example). In this event, a predator compares its most recent encounter to a fixed probability or template, and ignores its previous encounters. For the same example above, an encounter with a somewhat unpalatable butterfly (ß = 0.8) would elicit a future attack probability of 0.5 x 0.8 = 0.4, and after two encounters, the probability of the next attack would still be 0.5 x 0.8 = 0.4. The more unpalatable species (ß = 0.4) would elicit a reduced likelihood of predation of 0.5 x 0.2 = 0.1. Biologically, the function reflects constant, but lower inhibition with increases in unpalatability (or constant, but greater induction with increasing palatability).

The Continuum's Character

The Batesian-Müllerian continua, as discussed by Huheey and Turner, are fundamentally different in character. Huheey's treatment sees two equally unpalatable species neither gain nor suffer greater predation as a result of mimicry. Unequal palatability shifts the model so that members of the more-unpalatable lose at the expense of the less-protected. For Turner, two unpalatable species of equal palatability gain from mimicry. If unequal, both members still gain, but members of the unpalatable species (Model) gain less as the mimic (Mimic) becomes more palatable.

What is the evolutionary significance between the two mathematical models? In both cases a continuum is defined. At the extremes are the classical categories of Bates and Müller. In between these endpoints, however, lies the difference. For Huheey, the situation becomes more Batesian, for Turner less Müllerian. Thus, Huheey's continuum is under increasing negative frequency-dependent selection which leads to increasing pressures of color convergence/advergence. Turner's continuum is under decreasing positive frequency-dependent selection so that color patterns continue to converge, but more slowly for the more unpalatable species and more quickly for the less unpalatable one. The debate becomes, then, not is there a Batesian-Müllerian continuum in nature, but what is its character?

Huheey's Batesian-Müllerian continuum is based upon untested assumptions of competition and absolute abundance. For the ithomiines in Chapter 4, the likelihood that ithomiines compete and reduce each other's numbers is probably remote (see Vasconcellos-Neto, 1991). Turner's Batesian-Müllerian dichotomy results from similarly untested predator behavior and unchanging abundance. Further, both Turner and Huheey assume species-level palatability (i.e., they ignore automimicry). As noted by Fisher (1928) the precise nature of the Batesian-Müllerian continuum will not come from ignoring variation, but from incorporating it.

Costs of Chemical Defense

Both Huheey and Turner ignore the costs of chemical defense in their models. In fact, all models of chemical defense restrict their measures of fitness to predation risk. The cost of defense, though, is also an important component of fitness for potentially defended prey. A butterfly should only be defended, therefore, if the costs of that defense are more than compensated by reduced predation. Potential costs of chemical defense include the energetic costs of acquisition, the metabolic costs of sequestration, and the cost of autotoxicity (toxic effects experienced by the prey as a result of storing defensive compounds).

Costs further increase the benefits of mimicry to Batesian mimics, but would not alter the qualitative nature

of the interaction modelled here. For protected species, individuals lacking defense compounds (automimics) would have increased fitness through lower costs. A decrease in the proportion of protected individuals, however, would also decrease the benefits of mimicry in a frequency dependent manner. In addition, costs could also be frequency-dependent, as when insects compete for sources of defensive compounds. Species may also differ in the costs they accrue from chemical defense. If so, although benefits measured as reduced predation risk might be identical for different species, members of one may gain more from mimicry because of lower costs. Extremely high costs of chemical defense could preclude defense altogether.

Evidence for such costs is not strong (Bowers, 1988). Growth rates and adult size are not influenced by cardenolide content in several danaid species (Nymphalidae: Danainae), nor in male Euphydryas anicia (Nymphalidae: Nymphalinae). Cohen (1985) found a negative correlation between cardenolide concentration in larval food and some, but not all, larval weight and size parameters. Neither he nor Seiber et al. (1980) found correlations between larval cardenolide content and weight or size, however. Furthermore, E. anicia females show a positive correlation between iridoid glycoside content and weight (Bowers, 1988). In the following sections, therefore, I restrict my analyses only to the benefits of mimicry resulting from lowered predation pressure and, as with all mimicry models devised so far, assume no costs to

defense. Costs do not influence the ecological outcome of predator-prey interactions. However, cost remains an important consideration in the evolution of mimicry.

The Current Study

In this dissertation, I outline a novel approach to study variable chemical defense. The method begins once likely sources of defense have been identified. Given the chemical identity of potential defensive compounds, the operation of chemical defense in a group of organisms will be most effectively studied when researchers: (1) provide evidence that the potential chemical has negative pharmacological properties with sympatric predators, (2) show that the insects in question are protected when compounds are acquired and are not protected without them, (3) examine the naturally-occurring range and pattern of the chemical's quantitative variation, (4) determine a threshold amount that determines palatability, (5) assess the current influence of the variation, and (6) seek to explain future evolutionary directions resulting from the variation. In theory, this method should be applied to all chemically-defended species and should examine both the benefits and costs to chemical defense with mimicry (discussed earlier) and the response of predators to changing abundance (see below).

Here, I examined the first two steps with a single ithomiine species, outlined the pattern of variability for 20 ithomiine species that share a common class of protective alkaloids and color pattern, provided a threshold for a single predator, and assessed the current and future influence of variable defense with a mathematical model. The result was that ithomiine defense is based primarily upon adult-acquired pyrrolizidine alkaloids (larvae are poorly protected by some other group of compounds), pyrrolizidine alkaloids vary widely between individuals with a strong seasonal component, and ithomiine abundance and chemical defense interact to give a uniformly high level of protection against predators throughout the year.

The impact of predator foraging behavior on such data, unfortunately, cannot be examined with either Huheey's or Turner's mathematical treatments. While these models illustrate the basic importance of prey abundance, variable defense, and predator behavior to the character of the Batesian-Müllerian continuum, they cannot simultaneously examine within and between species variability in defense, demonstrated in Chapter 4. To do this, I once again use the Brower et al. (1970) model examined in Chapter 4.

The Continuum Revisited

The model by Brower et al. (1970) and the ithomiine data of Chapter 4 illustrated a surprising result that greatly

impacts the fundamental nature of Batesian and Müllerian mimicry. Results from Chapter 4 suggest that all species gain from the presence of a mimetic species, unpalatable or palatable, so long as the presence of one species does not decrease the population size of the others (as with competition). Additional survival occurs if the mimetic species contains some unpalatable individuals, but even a species composed of entirely palatable individuals will confer an advantage to a species with some defended members because of swamping effects. Thus, in direct opposition to Huheey, Turner, and classical mimicry theory, the Brower et al. (1970) model and data from Chapter 4 offer a third view of the Batesian-Müllerian debate: A continuum where everyone always benefits from mimicry but members of species benefit more (become increasingly Müllerian) with increasing unpalatability.

Here, as with Turner, Müllerian species (those with at least one unpalatable individual) gain from each other's presence. The degree of benefit will depend upon both their relative abundance and the proportion of protected individuals in each species: rare and less-protected species gain most. Unlike Huheey and Turner, however, members of protected species also gain from the presence of Batesian mimics. In this case, lower predation risk for members of the Müllerian species is solely the result of predator swamping.

The Assumptions

The result of the Brower et al. (1970) model is the old Batesian and Müllerian continuum but with a decidedly different character. Unlike Huheey's mathematical treatment, Müllerian species gain from each other's presence. Unlike Turner's, Müllerian species may also gain from the presence of Batesian species. The extent of the advantage is determined by both abundance and degree of variable chemical defense. The continuum extends from nearly no gain (addition of a rare palatable species) to one in which survival greatly increases in the presence of other species (addition of an abundant and well-protected species), and everything in between (e.g. Table 4-6).

The major assumption leading to this result is, again, the predator's foraging behavior. In this case, the predator does not respond to changes in abundance. While butterfly numbers double, the predation rate remains the same, so that each butterfly's probability of capture is one half its former value. The lack of a functional or numerical response on the part of the predator inherent in my use of the model makes ithomiine absolute abundance a principal component of their predation risk.

A lack of numerical response may occur with territorial species like the Blue-crowned Motmot (Momotus momota) used in the dissertation. Throughout the year, I saw only two of

these birds at any one time in the ithomiine assemblage. Motmots were site tenacious at other locations in the community, as well. A functional response on the part of Motmots, while expected, would have a small impact on predation pressure given the large changes in abundance found for tawny ithomiines in Monteverde for 1987. A Motmot that alters its foraging behavior from 1 to 30 butterflies eaten before satiation (in response to ithomiine abundance) would have been swamped by ithomiine numbers all months.

Of course, Motmots are not the only ithomiine predators in Monteverde. Numerical and functional responses of all ithomiine predators combined for Monteverde may significantly impact predation pressure and, therefore, the benefits of mimicry. I examine three ways in which predators can react to changes in prey density: (1) no change in response (UNCHANGING: predators forage without regard to prey abundance, as with Motmots modelled in Chapter 4), (2) increase predation in a linear fashion (TRACKING: a functional and/or numerical response that keeps predation risk to the average butterfly the same), or (3) increase predation to an exponential degree (APOSTATIC: predators increase foraging disproportionately in response to prey abundance, as when there is specialization and recruitment). The following sections re-examine the application of the Brower et al. (1970) model to the ithomiine data in Chapter 4 for these predator behaviors. I have explored the effects of the UNCHANGING predator in Chapter 4. In the following

sections, I adjust values of n and m from the model to simulate increased predation with increased abundance, approximating the effects of combined numerical and functional responses.

The Model

The Brower et al. (1970) model is outlined in Chapter 4. Briefly, it examines the likelihood of surviving an attack by first calculating the attack rate when all species are palatable:

$$Jo = 1 - n/m,$$

where Jo is the probability of survival, n is the number of butterflies a predator eats per unit time, and m is the number of butterflies present.

The model also allows one to calculate survivorship with variable defense. Here, the probability of survival is written as:

$$Jk' = 1 - [1 - (1 - k')^{n}/mk'),$$

where k' is the proportion of protected individuals in the population or assemblage. The difference between Jk' and Jo is written as ΔJ, or the mimetic advantage. This value

represents the increased likelihood of survival as a result of variable chemical defense.

Tracking Predation

The initial use of the Brower et al. (1970) model examines the situation when predators do not respond to changes in abundance. In this instance, there is neither a functional nor a numerical response. Does the outcome of the model change when predators adjust to changes in prey abundance to keep predation pressure the same? Here, I reexamine the Brower et al. (1970) model and simulate a tracking predator by keeping n/m a constant. That is, for every change in m (butterfly abundance) there is a corresponding change in n (number of butterflies eaten by predators). Thus,

$$n = ßm.$$

This would occur if one or more predators ate prey in proportion to prey abundance.

Apostatic Predation

Many predators have a functional response that is apostatic (Endler, 1991), that is they prey differentially on more common prey. Here, attack probabilities decline faster than prey density so that rare species are more protected than common ones. How, then, does the character of the

Batesian-Müllerian continuum change with an apostatic response?

To incorporate an apostatic predator into the Brower et al. (1970) model, I set n (number a predator eats per unit time) as:

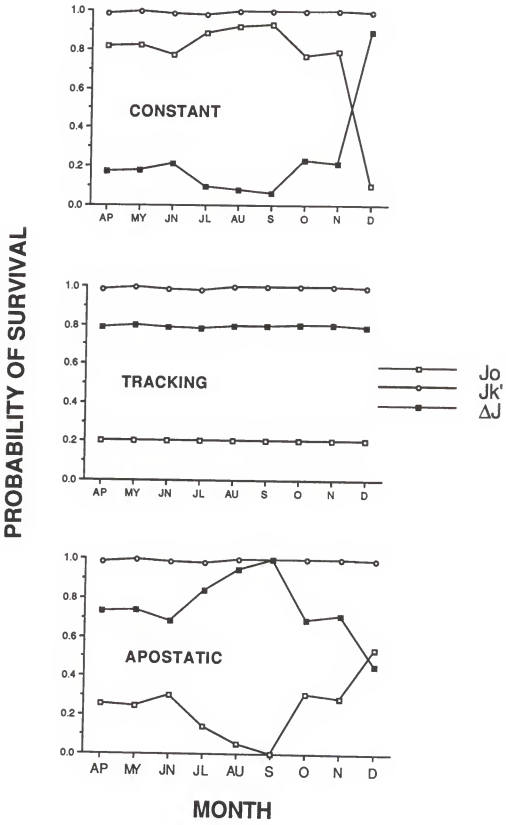
$$n = ßm^{\partial},$$

where ß and ∂ are constants. This is a function that allows n (number eaten) to increase faster than m (abundance of prey). It is understood that when n = m, n does not increase further.

Re-examination of Monthly Data from Chapter 4

Here, I take monthly values of combined tawny ithomiine abundance and variable defense and examine the outcome of mimicry to predation pressure each month. Here, though, I also include the impact of different foraging behaviors on the likelihood of tawny ithomiine survival. I selected values of n and ß that gave survival probability in April at or near 0.2. For this to occur, I chose the following relationships between predation pressure (n) and ithomiine abundance (m): (1) $n = 600$ (UNCHANGING), (2) $n = 0.8m$ (TRACKING), and (3) $n = 1.1m^{1.3}$ (APOSTATIC).

Figure 5-1. Probability of survival for the average tawny ithomiine in Monteverde, Costa Rica in 1987. Curves represent predation risk in the absence of chemical defense (Jo), with variable chemical defense (Jk'), and the difference between these (ΔJ, or the advantage of mimicry, measured as the increase in probability of survival resulting from variable defense with mimicry). Results are from data in Chapter 4 and the model of Brower et al. (1970). I present probability of survival for three conditions of predator response to changing ithomiine abundance: CONSTANT (no numerical nor functional response), TRACKING (linear functional and/or numerical response), and APOSTATIC (exponential functional and numerical response with changing prey abundance).


PROBABILITY OF SURVIVAL
1.0
0.8
0.6
0.4
0.2
0.0
CONSTANT
TRACKING
APOSTATIC
AP
MY
JN
JL
AU
S
O
N
D
MONTH
Jo
Jk'
ΔJ

Results for each month are given in Figure 5-1. These curves define changes in predation pressure in the absence of defense (Jo), with variable defense (Jk'), and the advantage of mimicry (ΔJ, the difference between Jk' and Jo). When predators are insensitive to changes in ithomiine abundance (UNCHANGING), risk of predation in the absence of defense decreases with increasing abundance. This occurs as prey swamp the effects of predators. When predators compensate for prey abundance in a linear fashion (TRACKING), predation pressure in the absence of defense is constant. However, when predation pressure increases disproportionately (APOSTATIC), predation pressure in the absence of defense is most intense at the highest ithomiine density.

Jo is sensitive to functional/numerical responses (it is defined as $1 - n/m$). Note, however, the impact of predation with variable defense (Jk') was uniformly low and did not change with predator behavior. That predation pressure does not alter Jk' may be understood by examining the formula for Jk' in more detail. As previously stated:

$$Jk' = 1 - [1 - (1 - k')^{n}/mk'),$$

where k' is the proportion of unpalatable individuals. I rewrite this value as:

$$Jk' = 1 - 1/mk' + (1 - k')^{n}/mk'.$$

The only term in this function that is sensitive to changes in predation pressure (n) is the last term:

$$(1 - k')^n/mk'.$$

Because $1 - k' < 1$, as n goes to infinity, this term rapidly becomes zero. As predation pressure increases, then, the probability of survival with variable defense becomes:

$$Jk' = 1 - 1/mk'.$$

Therefore, Jk' becomes independent of predation pressure (n). This was demonstrated in Figure 4-16 for the ithomiine data in Chapter 4. In most months, Jk' did not change above n = 40, and all months showed Jk' independent of predation pressure when n > 100. In addition, because m and k' are constant for each month, and m is usually high when k' is low, abundance continues to ameliorate the affect of variable defense (see Chapter 4). Even with increased predation pressure, then, there is a uniformly high level of protection for tawny ithomiines in Monteverde (shown in Figure 5-1).

The advantage of mimicry (ΔJ), therefore, is nearly a mirror image of predation in the absence of defense. Thus, with UNCHANGING predators mimicry is most beneficial to a tawny butterfly in Monteverde in months of low abundance, such as December, and is least advantageous in July, August and September. With TRACKING predators, however, mimetic

advantage is nearly constant. With APOSTATIC responses to ithomiine abundance, however, mimetic advantage was strongest in months of greatest ithomiine abundance. In Monteverde in 1987, this occurred from June to September. Mimetic advantage was lowest in December.

Explicitly testing the effects of foraging behavior on the operation of variable defense for tawny ithomiines in Monteverde has demonstrated: (1) probability of survival is largely independent of predation pressure when there is variable defense and (2) mimetic advantage changes fundamentally with changes in foraging strategy. Further, the probability of survival is independent of predation pressure for two reasons. When predation pressure is low, the swamping effect of relatively high ithomiine abundance results in low predator impact on survival. When predation pressure becomes great, variable defense makes ithomiine survival uniformly high. The advantage of mimicry to the average ithomiine, on the other hand, is nearly a mirror image of predation pressure. Mimetic advantage, in fact, cannot be measured without knowing the precise predator foraging behavior in the absence of chemical defense. In September, for example, mimetic advantage is either highest, lowest or the same as other months, depending on foraging behavior (Figure 5-1).

Abundance, Variable Defense, Foraging Behavior and the Evolution of Mimicry

The above example illustrates the importance of incorporating predator behavior into models of mimicry. The selective advantage of mimicry (measured as increased probability of survival), in fact, could not be assessed for ithomiines in Monteverde because I did not measure the potential foraging pressure on butterflies throughout the year. The following discussion extends the use of the Brower et al. model further. In this case, I use hypothetical data for just two species to determine how the character of the Batesian-Müllerian continuum changes with predation pressure.

I chose four hypothetical examples and the three paradigms of foraging behavior examined previously to illustrate the influence of abundance, variable defense, and predator foraging behavior on the character of the Batesian-Müllerian continuum. Conditions for the test of the model are in Table 5-1 as are the cost (negative numbers) or benefit (positive numbers) of mimicry (measured as $\Delta Jk'$). The results clearly show that predator behavior fundamentally changes the nature of mimicry between these species.

Batesian Mimicry

Batesian mimics always gain from mimicry. The addition of Batesian mimics is also advantageous to members of a protected species if predation pressure remains constant (unchanging predators). The benefit to members of the

protected species is a result of predator swamping. If predators compensate for changes in butterfly abundance (tracking predators) then there is no cost of Batesian mimics to members of protected species. In this case, dilution of protection is exactly compensated by predator swamping. Finally, individuals from protected species lose from Batesian mimicry if predators begin to consume disproportionate numbers of butterflies as this prey type becomes more common (apostatic predators).

Müllerian Mimicry: Equal Unpalatability

According to the Brower et al. model, Müllerian mimics always gain by their association. As predation pressure becomes more intense, however, the effect of swamping is removed and chemical defense becomes increasingly important. As a result, extreme predation pressure will cause the benefits of Müllerian mimicry to approach zero when each species is represented by the same proportion of unpalatable individuals.

Müllerian Mimicry: Unequal Palatability

Given equal abundance but unequal palatability, members of the least palatable species gain most from Müllerian mimicry. This trend is consistent with all predator foraging behaviors. Therefore, the Brower et al. model sees the Batesian-Müllerian continuum as becoming increasingly more Müllerian in character as species increase in unpalatability.

Table 5-1. Hypothetical Batesian-Müllerian benefits from mimicry. Mimetic advantage was measured using the model by Brower et al. (1970) for three foraging behaviors. Values for butterfly abundance (m) and proportion defended (k') are also reported.

Species	m	k'	Mimetic Advantage ΔJk' (Jk' together - Jk' species i) Unchanging	Tracking	Apostatic
Batesian Mimicry					
Protected	100	0.10	0.02	0.00	-0.01
Batesian	100	0.00	0.14	0.11	0.10
TOGETHER	200	0.05			
Müllerian Mimicry: equal unpalatability					
Species 1	100	0.05	0.06	0.04	0.03
Species 2	100	0.05	0.06	0.04	0.03
TOGETHER	200	0.05			
Müllerian Mimicry: unequal palatability					
Species 1	100	0.075	0.04	0.02	0.01
Species 2	100	0.025	0.09	0.07	0.06
TOGETHER	200	0.05			
Müllerian Mimicry, unequal abundance					
Species 1	150	0.05	0.02	0.02	0.02
Species 2	50	0.05	0.19	0.07	-0.01
TOGETHER	200	0.05			

Note: UNCHANGING (n = 20 butterflies eaten; predator does not respond to changing butterfly abundance), TRACKING (n = 0.2m; predator increases foraging effort linearly with increasing abundance), and APOSTATIC (n = $0.002m^2$; predator increases foraging exponentially with increasing abundance). These values set n = 20 at m = 100.

Müllerian Mimicry: Unequal Abundance

When two species are equally represented by unpalatable individuals, members of the least abundant species gain most from Müllerian mimicry when predation is constant (unchanging predators) or when predators exactly compensate for increased prey abundance (tracking predators). However, if predators forage preferentially on more common prey, members of the rare species actually are better off not resembling a more common, equally protected species. Herein lies a potential solution to the origin and evolution of "mimicry complexes," where several sympatric color patterns protect different mimetic species. Rare species remain distinct. Other rare species benefit from resembling other rare species, and so on. As species become established in the area they may increase population sizes and result in two different, equally abundant color complexes in a single location.

The character of the Batesian-Müllerian continuum, then, is variable and depends in part on how predators react to changes in prey abundance. Batesian mimicry may benefit, cost, or have no impact on members of a protected species. Members of Müllerian species gain most from mimicry when they are from rare or poorly-protected species. As species decrease in the proportion of protected individuals they contain, members of the other species gain less. Finally, for members of rare species escaping apostatic predation,

Müllerian mimicry with an equally-protected but more abundant species comes at a cost.

Re-examination of Ithomiines in December

Of course, the above trends are further complicated by simultaneous changes in abundance and degree of defense. The data for ithomiines in December provide an illustration of this (Table 5-2). For tawny ithomiines in Monteverde, the outcome of a tracking predator is the Batesian-Müllerian continuum of Turner. Chemically-defended species always benefit from mimicry. The extent of the mimetic advantage ($\Delta Jk'$), depends upon the proportion of protected individuals the species represents (i.e., members of species with a greater proportion of protected individuals gain least from mimicry). Abundance is still important, however. For instance, I. xenos and P. fulvescens have similar k' but I. xenos is much more abundant. Their predation pressures when both are palatable are identical at 0.9. With nearly the same degree of variable defense, however, I. xenos suffers less predation, 0.99 survivorship compared to 0.925 for P. fulvescens. This difference is the result of extremely low abundance in P. fulvescens. Birds will sample one tenth, or 1.7 P. fulvescens, compared to 10.6 I. xenos. The likelihood of sampling an unpalatable butterfly in these two cases is greater for I. xenos (as the predator has 11 attempts, rather than just 2).

The result of apostatic predation supports the classical ideas of Müllerian and Batesian mimicry, but favors rare species. As abundance increases, unpalatability and automimicry are increasingly effective anti-predator strategies. All species lose by association in the absence of chemical defense (all ΔJo were negative because predation increased disproportionately with increased abundance). Müllerian mimicry increases protection for all species involved, but is most important for rare species. In this case, the rare species was also well-protected. According to this treatment of the Brower et al. model, the impact of a Batesian mimic is to decrease the survival of variably-defended species. Further, although not explicitly shown, the extent of the cost will be highly frequency-dependent. The hypothetical Batesian species decreases the average *I. xenos*' fitness in two ways: it decreases the overall degree of chemical protection while simultaneously increasing predation pressure through increased prey abundance.

Conclusions

This dissertation firmly asserts the need to incorporate automimicry and predator behavior into discussions and mathematical treatments of mimicry. Evolutionarily important distinctions between Batesian and Müllerian mimicry will not be answered with typological treatment. This dissertation develops a method for studying chemical defense that begins

by demonstrating the protective nature of naturally-occurring compounds in animals. It extends this knowledge greatly by attempting to determine thresholds and apply them to patterns of variable defense. For large numbers of mimetic species, the significance of variable defense can be assessed within existing models of automimicry. The source of the variation is also explored here and is a combination of differential use and acquisition between species, and seasonal availability of compounds used in defense.

As it turns out, the significance of all these factors cannot be fully assessed without knowing if mimetic species influence each other's abundance, and the precise nature of a predator's foraging behavior (see Malcolm, 1990). I did not measure the behavior of Spiny Lizards or Blue-crowned Motmots to changing ithomiine abundance. Therefore, the assessment of variable defense discussed in the present chapter and in Chapter 4 remains somewhat hypothetical. These factors determine not just the degree of convergence, divergence, or advergence between species, but the quality of color pattern evolution, as well. Qualitative differences in defensive chemistry may also be important. Finally, the costs of chemical defense should be documented. If significant, models need to incorporate both cost and benefit of a chemically mediated defense strategy.

In the end, the dissertation affirms the quote by Fisher at the beginning of this chapter. The variable nature of color pattern evolution between species in a mimetic

Table 5-2. Type of predator foraging behavior alters the relative survivorship of tawny ithomiines from Monteverde, Costa Rica in December 1987.

Species	m	k'	J_0	Jk'	ΔJ	ΔJ_0	$\Delta J_{k'}$
UNCHANGING PREDATOR							
I. xenos	106	0.68	0.81	0.99	0.17	0.07	0.003
I. heraldica	43	0.20	0.53	0.88	0.35	0.35	0.104
P. fulvescens	17	0.67	0.00	0.91	0.91	0.88	0.090
Assemblage	166	0.55	0.88	0.99	0.11	-	-
TRACKING PREDATOR							
I. xenos	106	0.68	0.90	0.99	0.09	0.00	0.003
I. heraldica	43	0.20	0.90	0.93	0.03	0.00	0.061
P. fulvescens	17	0.67	0.90	0.93	0.25	0.00	0.063
Assemblage	166	0.55	0.90	0.99	0.09	-	-
APOSTATIC PREDATOR							
I. xenos	106	0.68	0.84	0.99	0.14	-0.01	0.003
I. heraldica	43	0.20	0.85	0.91	0.06	-0.02	0.080
P. fulvescens	17	0.67	0.87	0.92	0.05	-0.03	0.100
Assemblage	166	0.55	0.83	0.99	0.16	-	-

Note: The UNCHANGING PREDATOR ignores changes in abundance and eats 20 ithomiines per unit time. The TRACKING PREDATOR increases foraging effort on tawny ithomiines in a linear fashion with ithomiine abundance, so that $n = 0.1m$ (n = number of ithomiines eaten per unit time; m = ithomiine abundance). The APOSTATIC PREDATOR forages more heavily on ithomiines as they become abundant, so that $n = 0.1m^{1.1}$. Probability of survivorship is measured using the model by Brower et al. (1970), where jo is mean survivorship in the absence of chemical defense and jk' is survivorship when k' individuals are protected. Automimetic advantage is ΔJ = jk' - jo. The net effect of joining a mimetic assemblage without defense is ΔJo = jo assemblage - jo $species_i$. In the presence of variable defense this becomes $\Delta Jk'$ = jk' assemblage - jk' $species_i$.

relationship is so diverse that Batesian and Müllerian categories are effectively reduced to textbook examples and probably do not naturally exist in their pure state for most species. Rather, at any given point in time species are, to one degree or another, more Batesian or more Müllerian. Yet, it appears that mimicry operates in spite of such variation, as evidenced by the striking similarity in color pattern found in ithomiines and many other species. If mimicry explains this coincident coloration, it is, indeed, a powerful example of evolution by natural selection. That mimicry theory continues to allow clear analyses of evolutionary forces in the face of increasing sources of variation is a testament to its ongoing usefulness as a premier tool to study adaptation.

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BIOGRAPHICAL SKETCH

I was born October 15, 1959, in Chicago, IL to Wayne L. and JoAnn M. Masters. Unlike most other ecologists I know, I was largely denied access to the natural world until adulthood. I can honestly say that this has been my only regret. I believe my childhood is an example of a common malaise: how a child lives wholly separate from nature. Happily, my life also demonstrates a way out. Through my formal education, I have developed a relationship with the natural world that is a great source of happiness and contentment. The following sketch relates events I believe led me to the completion of this dissertation and a life dedicated, in part, to understanding the environment.

When I was two years old, my parents moved to Iowa. They built a new home, exposing me to the beauty of nearby fallow fields. My brother, Brian, and I spent long days before school age making gum out of milkweed latex, catching butterflies, and keeping lightning bugs in jars. I clearly remember the thistle and milkweed attracting large numbers of butterflies. Throughout our preschool nature study, we were never encouraged, never told the names of organisms, never shown pattern or process, nor did we care. In time, the

field became several homes and our natural toys were replaced by plastic guns, frisbees, and T.V.

When I was eight years old we moved to the suburbs of Chicago where forest was restricted to a narrow strip along the Des Plaines river. Despite my mother's protests, I braved elements of violent crime and illicit substance use in these forest preserves to view the occasional deer or mature tree. I remember enjoying the local nature center. In sixth grade, the 13 year cicadas came out in huge numbers. I clipped articles about them from the newspaper, saved their cast cuticles, and rescued the occasional victim of impending ant predation. Later, I was covered by poison ivy welts over 90 % of my body.

I attended John Hersey High School from 1974 to 1978. There were a tremendous number of biology courses that I attended, mostly human oriented. I believed I would become a doctor and so dutifully learned bones and major muscle groups The majority of my free time and most energy were spent in movie theaters, bowling alleys and restaurants. Friends and I went to Canada to canoe in Quetico Primitive Wilderness Area during two summers. We were mostly glad to be independent, but saw moose and pulled a few leeches from our ankles. Despite high scores, I was denied a letter of recommendation by a favorite teacher because of an "attitude problem." This convinced me to validate my intelligence, knowledge, and willingness to learn in an academic manner.

In 1978, I was accepted to Illinois State University, where I graduated in 1982 cum laude in biological sciences with a minor in chemistry. While there, I enrolled in general ecology with Dr. Boyce A. Drummond III, an ithomiine biologist. In his course, we managed to study what little woods were left between corn and soybean. My junior and senior years, I worked for several ecologists at Illinois State University. I had found a few more remnants of natural habitat.

I came to the University of Florida in 1982. In January the following year, I spent one month in Mexico at a monarch overwintering aggregation. Research performed there with Dr. L.P. Brower eventually became a master's thesis in zoology, awarded in December 1985. I spent the summer of 1983 in Costa Rica with twenty students and the Organization for Tropical Studies. Dr. William A. Haber was a coordinator and ithomiine biologist, also. He greatly encouraged me to pursue a dissertation research project on ithomiine biology. Upon returning to Florida, Dr. Brower agreed to a study of ithomiine chemical defense.

The following summer was profound. I returned alone to Costa Rica to begin ithomiine research. It was at this time that I began independent research. That same summer, I met Karen Hoffmann. Karen and I explored Costa Rica together. We shared interesting natural history observations with one another between theme songs from The Brady Bunch and Partridge Family. Here was another soul caught between a

society that denied the importance of nature, experiencing the natural world's beauty for the first time.

By the time I returned to Florida, I loved both tropical biology and Karen. Karen and I married in Costa Rica in 1988 while I gathered data for this dissertation. We became parents five months later. The identical twins were named Cameron and Adrian.

The boys are nearly four and, through them, Karen and I are discovering the wonders of temperate biology in Princeton, NJ. The fallow fields are getting increasingly difficult to find, the woods are smaller and farther apart now, but we actively seek out what little is left. Karen and I are proud and happy to share our recent knowledge of ecology with our children. Together, I hope we build a familial heritage of nature study.

As our world deforests and further denounces the worth of nonhuman life, as people increasingly sever what few ties to nature remain, our family will join the many people dedicated to exploring nonhuman biology and teaching it to others. This dissertation is a fundamental step. I hope it leads to a life surrounded by nature, with the formal training necessary to approach nature experimentally, the soul to see it metaphysically, and the credentials to tell others what I have learned.

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Lincoln P. Brower, Chair
Professor of Zoology

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Thomas C. Emmel
Professor of Zoology

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

H. Jane Brockmann
Professor of Zoology

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Thomas J. Walker
Professor of Entomology
and Nematology

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Robert K. Vander Meer
Assistant Professor of
Entomology and
Nematology

This disseration was submitted to the Graduate Faculty of the Department of Zoology in the College of Liberal Arts and Sciences and to the Graduate School and was accepted as partial fulfillment of the requirements for the degree of Doctor of Philosophy.

August, 1992

Dean, Graduate School